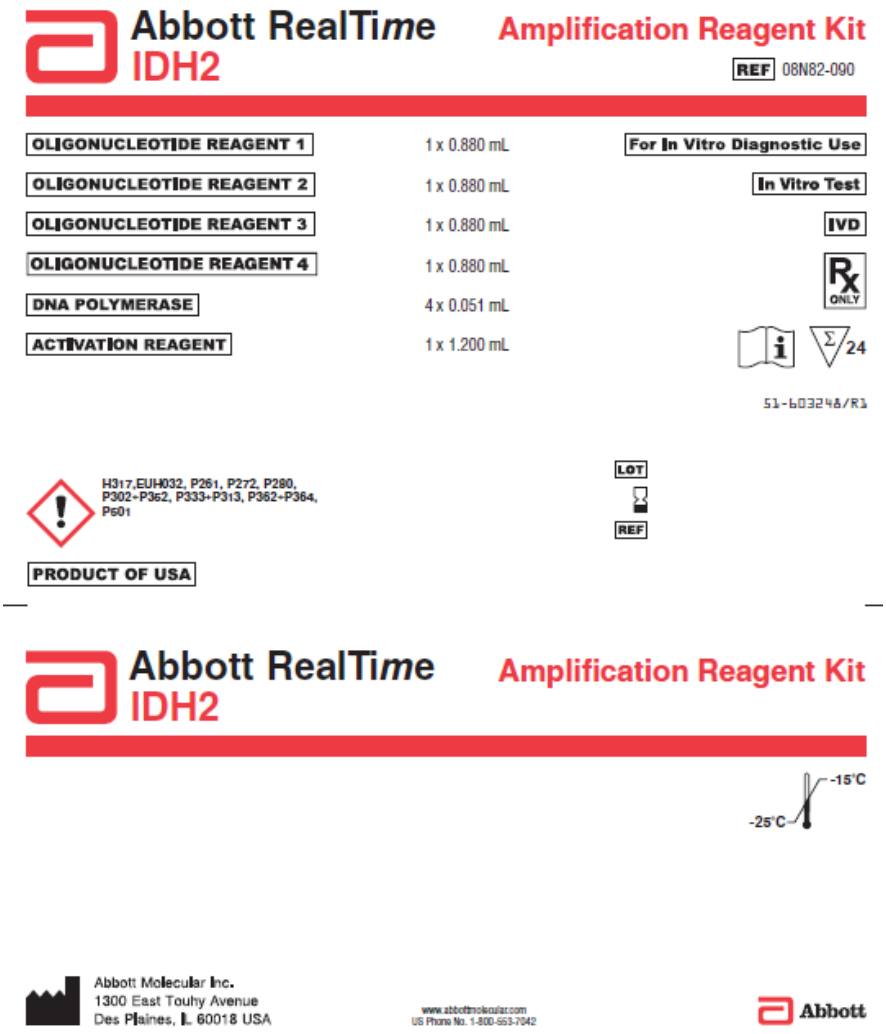
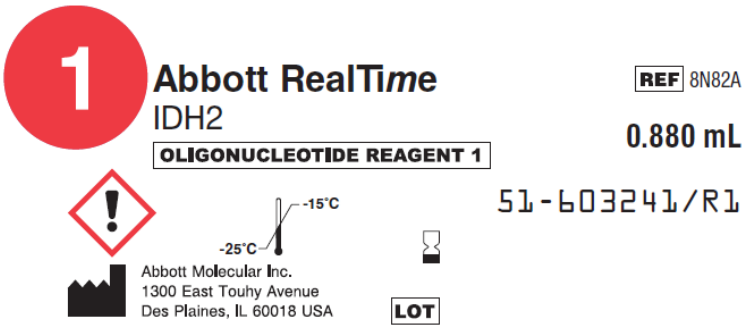
1.0 Abbott RealTi*me* IDH2 Labeling

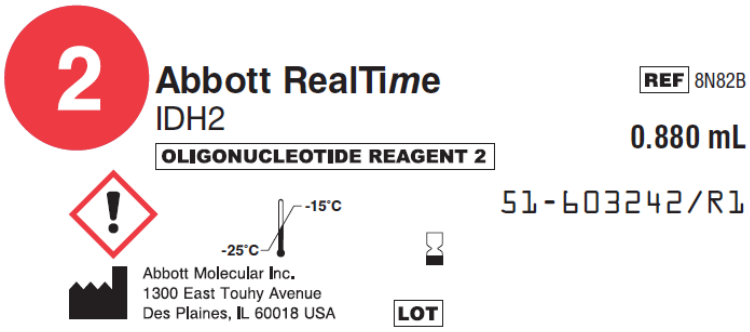
1.1 Abbott RealTi*me* IDH2 Amplification Reagent Kit Label



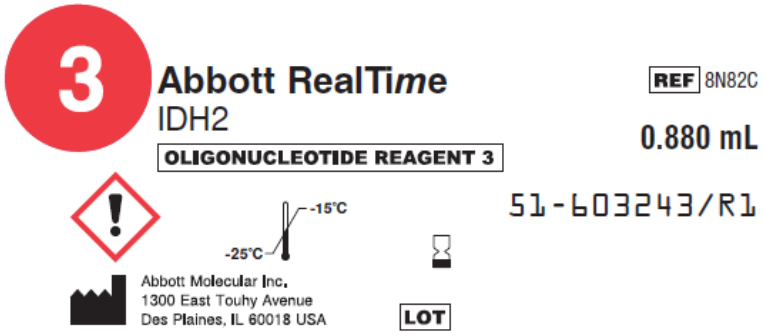
1.2 Abbott RealTi*me* IDH2 Oligonucleotide Reagent 1 Label



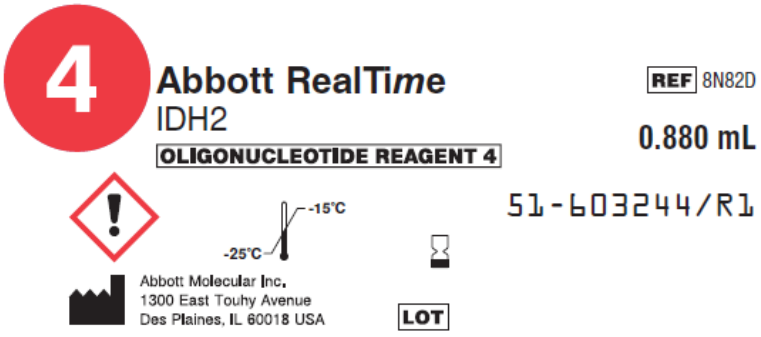
1.3 Abbott RealTi*me* IDH2 Oligonucleotide Reagent 2 Label



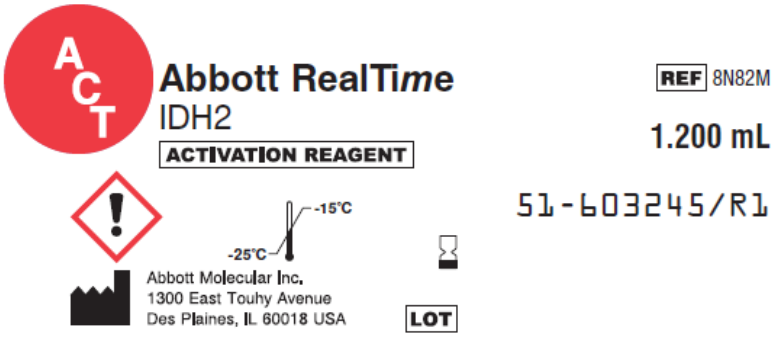
1.4 Abbott RealTi*me* IDH2 Oligonucleotide Reagent 3 Label



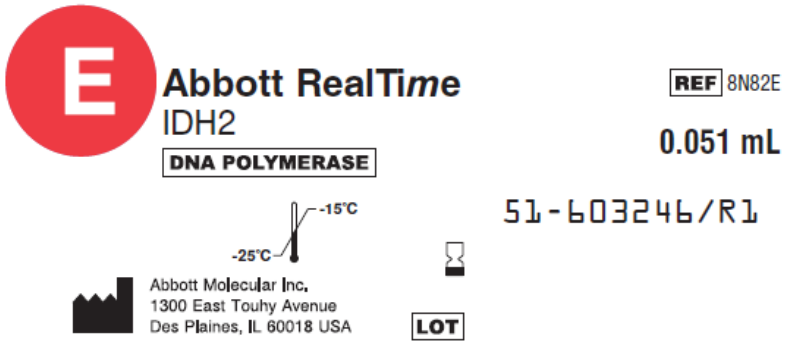
1.5 Abbott RealTi*me* IDH2 Oligonucleotide Reagent 4 Label



1.6 Abbott RealTi*me* IDH2 Activation Reagent Label

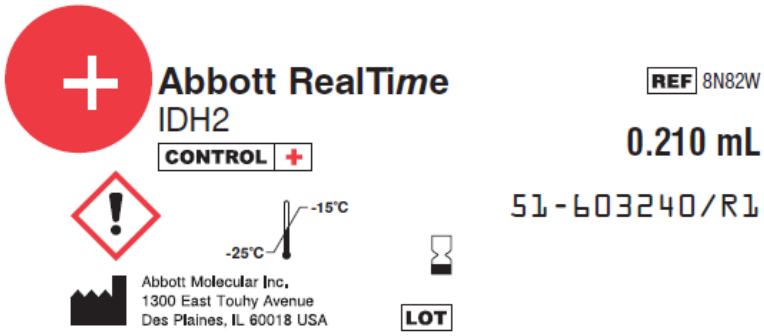


1.7 Abbott RealTi*me* IDH2 DNA Polymerase Label

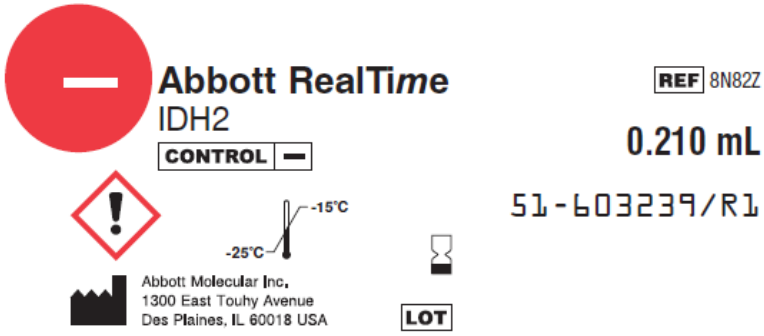


1.8 Abbott RealTi*me* IDH2 Control Kit Label

1.9 Abbott RealTi*me* IDH2 Positive Control Label



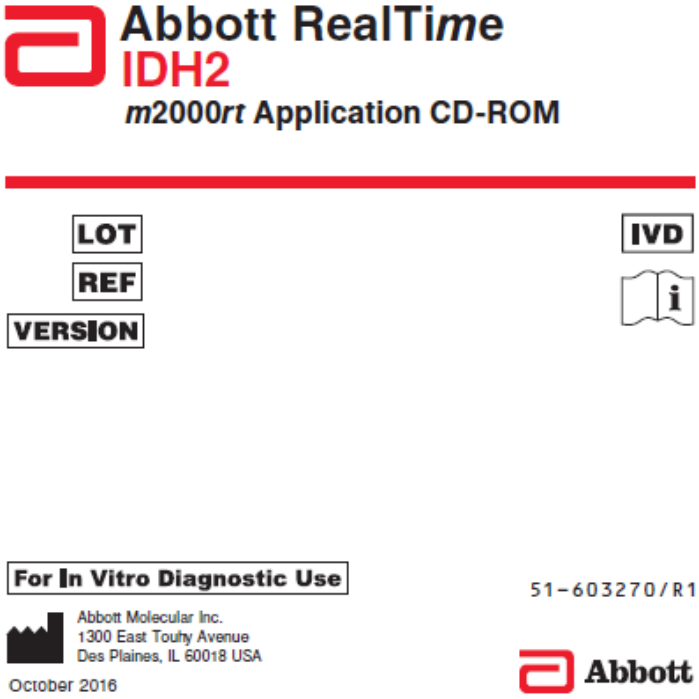
1.10 Abbott RealTi*me* IDH2 Negative Control Label



1.11 Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM Disc Label

51-603269/R1

1.12 Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM Carton Label



Abbott RealTi*me* IDH2

*m*2000*rt* Application CD-ROM Version 1.0

IVD

REF 08N82-001

51-603271/R1

REF 08N82-001

51-603271/R1

Key to Symbols Used

REF	Reference Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
VERSION	Version
i	Consult instructions for use
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
	Manufacturer

Please read the following information prior to installing the Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM, Version 1.0 on your *m*2000*rt* System.

Intended Use

The Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM, Version 1.0 (List No. 08N82-001) is intended for use with the Abbott Realti*me* IDH2 Assay (List No. 08N82) on the *m*2000 Instrument. Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM, Version 1.0 (List No. 08N82-001) contains the RealTi*me* IDH2 assay application files that can be installed for use on the Abbott *m*2000*rt* System.

The following are important facts to know before installing this Assay CD-ROM version:

- The Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM, Version 1.0 can only be used with *m*2000*rt* System Software Version 8.0 and higher.
- To install the Application Specification Files located on the Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM, Version 1.0, follow the instructions found in section 5 of the *m*2000*rt* System Operations Manual under Application Management.
- Once the CD-ROM has been placed in the CD-ROM drive, allow two minutes for the drive to read the CD-ROM before proceeding with the installation instructions. If the CD-ROM will not read, remove and reseat the CD-ROM in the CD-ROM drive and again allow two minutes to read.

Assay Specific Information

Application Name	Version	File Path Name
RealTi*me* IDH2	1.00	d:\AppSpecificationFiles\RealTime_IDH2_v1.0.asy

Host Configuration
For Configuration of your host, please refer to the following table.

Assay Name	Assay ID
IDH2	IDH2

Important: Please retain this document in your *m*2000*rt* System Operations Manual for documentation of assay file versions available on this CD-ROM.

For additional technical assistance, please contact your Area Customer Support.

Abbott Molecular Inc.
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www.abbottmolecular.com
October 2016
51-603271/R1

Abbott

Abbott RealTi*me* IDH2 Control Kit

en

REF 08N82-080
51-608431/R1

REF 08N82-080
51-608431/R1

Key to Symbols Used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
PRODUCT OF USA	Product of USA
Rx ONLY	Prescription Use Only
	Use by
CONTROL –	Negative Control
CONTROL +	Positive Control
	Temperature Limit
	Consult instructions for use
	Warning
	Manufacturer

CUSTOMER SERVICE: 1-800-553-7042

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

Abbott RealTi*me* IDH2 Control Kit

INTENDED USE

The Abbott RealTi*me* IDH2 Controls are used to establish run validity of Abbott RealTi*me* IDH2 when used for the qualitative detection of single nucleotide variants (SNVs) coding nine IDH2 mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) in DNA extracted from human blood (EDTA) or bone marrow (EDTA).

REAGENTS

Abbott RealTi*me* IDH2 Control Kit

(List No. 08N82-080)

1. CONTROL – **Abbott RealTi*me* IDH2 Negative Control (List No. 8N82Z) (5 vials, 0.210 mL per vial).** <0.01% noninfectious plasmid DNA in a buffered solution with carrier DNA. Preservatives: sodium azide and 0.15% ProClin® 950.
2. CONTROL + **Abbott RealTi*me* IDH2 Positive Control (List No. 8N82W) (5 vials, 0.210 mL per vial).** <0.01% noninfectious plasmid DNA in a buffered solution with carrier DNA. Preservatives: sodium azide and 0.15% ProClin 950.

The Abbott RealTi*me* IDH2 Control Kit must only be used with the Abbott RealTi*me* IDH2 Amplification Reagent Kit (List No. 08N82-090).

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

For In Vitro Diagnostic Use

- Consult Abbott RealTi*me* IDH2 package insert instructions for use.
- Do not use beyond expiration date.
- The CONTROL – and CONTROL + vials are intended for single use only; remaining reagents after first use should be discarded.

The controls contain sodium azide and 2-methyl-2H-isothiazol-3-one, which is a component of ProClin 950.

Abbott RealTi*me* IDH2 Negative Control

Abbott RealTi*me* IDH2 Positive Control

The following warnings apply:

Warning	
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
P261	Avoid breathing mist / vapours / spray.
P280	Wear protective gloves / protective clothing / eye protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

STORAGE INSTRUCTIONS

-15°C -25°C The Abbott RealTi*me* IDH2 Control Kit must be stored at −25 to −15°C.

SHIPPING CONDITIONS

The product is shipped on dry ice.

If assay reagents are received in a condition contrary to the label recommendation, or are damaged, contact Abbott Technical Services.

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 or visit the Abbott Molecular website at http://www.abbottmolecular.com.

Abbott RealTi*me* is a trademark of Abbott Laboratories.
All other trademarks are property of their respective owners.

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Des Plaines, IL 60018 USA

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Abbott

Abbott RealTi*me* IDH2

en

REF 08N82-090

51-608430/R1

REF 08N82-090

51-608430/R1

Rx ONLY

Key to Symbols Used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
PRODUCT OF USA	Product of USA
Rx ONLY	Prescription Use Only
	Use by
CONTROL –	Negative Control
CONTROL +	Positive Control
Σ	Contains sufficient for <n> tests
	Temperature Limit
	Consult instructions for use
	Warning
	Manufacturer
OLIGONUCLEOTIDE REAGENT 1	Oligonucleotide Reagent 1
OLIGONUCLEOTIDE REAGENT 2	Oligonucleotide Reagent 2
OLIGONUCLEOTIDE REAGENT 3	Oligonucleotide Reagent 3
OLIGONUCLEOTIDE REAGENT 4	Oligonucleotide Reagent 4
DNA POLYMERASE	DNA Polymerase
ACTIVATION REAGENT	Activation Reagent

CUSTOMER SERVICE: 1-800-553-7042

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

Abbott RealTi*me* IDH2

INTENDED USE

Abbott RealTi*me* IDH2 is an *in vitro* polymerase chain reaction (PCR) assay for the qualitative detection of single nucleotide variants (SNVs) coding nine IDH2 mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) in DNA extracted from human blood (EDTA) or bone marrow (EDTA). Abbott RealTi*me* IDH2 is for use with the Abbott *m*2000*rt* System.

INDICATION FOR USE

Abbott RealTi*me* IDH2 is indicated as an aid in identifying acute myeloid leukemia (AML) patients with an isocitrate dehydrogenase-2 (IDH2) mutation for treatment with IDHIFA® (enasidenib).

This test is for prescription use only.

SUMMARY AND EXPLANATION OF THE TEST

Abbott RealTi*me* IDH2 detects single nucleotide variants (SNVs) coding nine IDH2 mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) by using PCR technology with homogeneous real-time fluorescence detection. The assay uses human blood or bone marrow aspirate specimens and reports a qualitative result. **Table 1** lists the IDH2 mutations detected by the Abbott RealTi*me* IDH2 assay.

Table 1. Mutations Detected by the Abbott RealTi*me* IDH2 Assay

Codon	IDH2 Mutation	SNV
R140	R140Q	CAG
	R140L	CTG
	R140G	GGG
	R140W	TGG
R172	R172K	AAG
	R172M	ATG
	R172G	GGG
	R172S	AGT and AGC
	R172W	TGG

IDH2 (isocitrate dehydrogenase-2) is a mitochondrial enzyme that helps break down nutrients and generate energy for the cell. The homodimeric enzyme catalyzes a reaction that converts isocitrate to α-ketoglutarate (α-KG) while reducing NADP to NADPH and liberating CO_2. Because of its involvement in cellular energy production, IDH2 plays a role in the metabolism of glucose, fatty acids, and glutamine; and also contributes to the maintenance of normal cellular redox status.[1]

Mutations in the R140 and R172 codons of IDH2 are oncogenic and can be found in several cancer types, including acute myeloid leukemia (AML) in which 8% to 19% of patients possess an IDH2 mutation.[1,2,3,4,15] Nine IDH2 amino acid changes have been identified in various cancer types: R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172W, and R172S.[3,5,6,7] R140 mutations account for 80% of IDH2 mutations, with R140Q being the most prevalent, occurring in 30% to 50% of AML patients with IDH2 mutations.[5,6,15] Mutations in R140 and R172 are associated with the ability of IDH2 to further process α-KG to generate 2-hydroxygluturate (2-HG), an oncometabolite, which can result in concentrations that are orders of magnitude higher than normal.[8,9,10,11,12,13] Once produced, 2-HG alters the cells' genetic programming, or epigenetics, resulting in increased numbers of quickly proliferating, early hematopoietic progenitor cells, and tumorigenesis.[12,14]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Abbott RealTi*me* IDH2 consists of two kits:

- Abbott RealTi*me* IDH2 Amplification Reagent Kit (List No. 08N82-090)
- Abbott RealTi*me* IDH2 Control Kit (List No. 08N82-080)

Specimens for Abbott RealTi*me* IDH2 are processed manually using Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24) reagents to isolate and purify sample DNA. The Abbott RealTi*me* IDH2 amplification

reagents are combined into four amplification master mixes. The purified DNA sample is combined with the master mixes in an Abbott 96-Well Optical Reaction Plate, and the plate is transferred to the Abbott *m*2000*rt* instrument for amplification and detection of IDH2 mutations. The specimen result is automatically reported on the Abbott *m*2000*rt* workstation at run completion. Assay controls are included within each run and are processed through the DNA extraction, amplification, and detection steps of the assay to assess run validity.

Software parameters specific to Abbott RealTi*me* IDH2 are contained in an assay application specification file, which is loaded onto the Abbott *m*2000*rt* instrument by using a CD-ROM disk.

DNA Extraction

The purpose of DNA extraction is to isolate and purify genomic DNA from EDTA preserved blood or bone marrow aspirate specimens to make it accessible for amplification and to remove potential inhibitors of amplification. This process is accomplished by using the Abbott *m*Sample Preparation System$_{DNA}$ Kit, which uses magnetic particle technology to isolate and purify DNA. During the DNA extraction procedure, cells are lysed at an elevated temperature in a lysis buffer containing guanidine isothiocyanate. DNA is captured on magnetic microparticles, and inhibitors are removed by performing a series of washes with wash buffers. The bound DNA is eluted from the microparticles with elution buffer and is ready for PCR amplification.

Reagent Preparation and Reaction Plate Assembly

The Abbott RealTi*me* IDH2 Oligonucleotide Reagents (Oligonucleotide Reagent 1, Oligonucleotide Reagent 2, Oligonucleotide Reagent 3, and Oligonucleotide Reagent 4) are each manually combined with DNA Polymerase, and Activation Reagent to create 4 unique master mixes. These master mixes are added to 4 separate wells of the Abbott 96-Well Optical Reaction Plate with aliquots of the extracted DNA sample. After manual application of the Abbott Optical Adhesive Cover, the plate is transferred to the Abbott *m*2000*rt* instrument.

Amplification/Detection

Each Abbott RealTi*me* IDH2 master mix is designed to amplify and detect 2 or 3 IDH2 amino acid mutations (codon with mutant nucleotide underlined). Oligonucleotide 1 master mix amplifies and detects R140Q (C<u>A</u>G) and R140L (C<u>T</u>G). Oligonucleotide 2 master mix amplifies and detects R140G (<u>G</u>GG) and R140W (<u>T</u>GG). Oligonucleotide 3 master mix amplifies and detects R172K (A<u>A</u>G) and R172M (A<u>T</u>G). Oligonucleotide 4 master mix amplifies and detects R172S (AG<u>T</u> and AG<u>C</u>), R172G (<u>G</u>GG), and R172W (<u>T</u>GG). Refer to **Table 2**. In addition, each master mix is designed to amplify and detect a region of the IDH2 gene outside of codon 140 and 172, which serves as an endogenous internal control (IC).

Table 2. IDH2 Mutations Detected by each Master Mix

Master Mix	IDH2 Mutation	SNV
Oligonucleotide 1	R140Q	C<u>A</u>G
	R140L	C<u>T</u>G
Oligonucleotide 2	R140G	<u>G</u>GG
	R140W	<u>T</u>GG
Oligonucleotide 3	R172K	A<u>A</u>G
	R172M	A<u>T</u>G
Oligonucleotide 4	R172G	<u>G</u>GG
	R172S	AG<u>T</u> and AG<u>C</u>
	R172W	<u>T</u>GG

During the amplification reaction on the Abbott *m*2000*rt* instrument, the target DNA is amplified by DNA Polymerase in the presence of primers, deoxyribonucleoside triphosphates (dNTPs), and magnesium chloride ($MgCl_2$). The DNA Polymerase used in the assay is a thermophilic enzyme that has been chemically modified rendering it inactive.

During the amplification reaction of Abbott RealTi*me* IDH2, DNA Polymerase is first activated at a high temperature. During each subsequent round of thermal cycling, a high temperature is used to melt double-stranded DNA strands, followed by a low temperature where primers anneal to their respective targets and are extended to generate double-stranded DNA products. Exponential amplification of the products is achieved through repeated cycling between high and low temperatures. Amplification of IDH2 mutation and IC targets takes place simultaneously in the same PCR well.

IDH2 products are detected during the annealing/extension step by measuring the real-time fluorescence signals of the IDH2 mutation and IC-specific probes, respectively. The IDH2 mutation and IC-specific probes are labeled with different fluorophores, allowing their signals to be distinguishable in a single PCR well.

Assay Results

For each patient sample, 4 PCR reactions are evaluated. Abbott RealTi*me* IDH2 is a qualitative assay for which specimen interpretations are reported as "Mutation Detected" or "Not Detected." For specimens with interpretations of "Mutation Detected", the identity of the IDH2 mutation detected is reported. Refer to the **INTERPRETATION OF RESULTS** section for further details.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- Abbott RealTi*me* IDH2 performs amplification and fluorescence detection in a sealed Abbott 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the 96-Well Optical Reaction Plate.
- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- Separate dedicated areas are used to perform Abbott RealTi*me* IDH2. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

Abbott RealTi*me* IDH2 Amplification Reagent Kit (List No. 08N82-090)

1. **OLIGONUCLEOTIDE REAGENT 1** **Abbott RealTi*me* IDH2 Oligonucleotide Reagent 1 (List No. 8N82A) (1 vial, 0.880 mL)**

   < 0.1% synthetic oligonucleotides and < 1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin® 950.

2. **OLIGONUCLEOTIDE REAGENT 2** **Abbott RealTi*me* IDH2 Oligonucleotide Reagent 2 (List No. 8N82B) (1 vial, 0.880 mL)**

   < 0.1% synthetic oligonucleotides and < 1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

3. **OLIGONUCLEOTIDE REAGENT 3** **Abbott RealTi*me* IDH2 Oligonucleotide Reagent 3 (List No. 8N82C) (1 vial, 0.880 mL)**

   < 0.1% synthetic oligonucleotides and < 1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

4. **OLIGONUCLEOTIDE REAGENT 4** **Abbott RealTi*me* IDH2 Oligonucleotide Reagent 4 (List No. 8N82D) (1 vial, 0.880 mL)**

   < 0.1% synthetic oligonucleotides and < 1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

5. **DNA POLYMERASE** **Abbott RealTi*me* IDH2 DNA Polymerase (List No. 8N82E) (4 vials, 0.051 mL per vial)**

   DNA Polymerase (5.4 to 5.9 Units / µL) in a buffered solution with stabilizers.

6. **ACTIVATION REAGENT** **Abbott RealTi*me* IDH2 Activation Reagent (List No. 8N82M) (1 vial, 1.200 mL)**

   50 mM magnesium chloride in a buffered solution. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

Abbott RealTi*me* IDH2 Control Kit (List No. 08N82-080)

1. **CONTROL +** **Abbott RealTi*me* IDH2 Positive Control (List No. 8N82W) (5 vials, 0.210 mL per vial)**

   < 0.01% noninfectious plasmid DNA in a buffered solution with carrier DNA. Preservatives: sodium azide and 0.15% ProClin 950.

2. **CONTROL –** **Abbott RealTi*me* IDH2 Negative Control (List No. 8N82Z) (5 vials, 0.210 mL per vial)**

   < 0.01% noninfectious plasmid DNA in a buffered solution with carrier DNA. Preservatives: sodium azide and 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

For In Vitro Diagnostic Use

Abbott RealTi*me* IDH2 is for use with EDTA preserved human blood and EDTA preserved bone marrow aspirate.

Use only USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to prepare the *m*Wash 2_{DNA} sample preparation reagent. **Do not use ethanol that contains denaturants**.

Safety Precautions

Refer to the Abbott *m*2000*rt* Operations Manual, **Hazards** section, for instructions on safety precautions.

The Abbott RealTi*me* IDH2 Oligonucleotide Reagent 1, Oligonucleotide Reagent 2, Oligonucleotide Reagent 3, Oligonucleotide Reagent 4, Activation Reagent, Positive Control, and Negative Control contain the following components:

- 2-Methyl-2H-isothiazol-3-one
- Sodium azide

The following warnings apply:

Warning

H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
P261	Avoid breathing mist / vapours/spray.
P280	Wear protective gloves / protective clothing/eye protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product and the Abbott *m*Sample Preparation $System_{DNA}$ kit is contained in the Safety Data Sheets.

SPECIAL PRECAUTIONS

Abbott RealTi*me* IDH2 is for use with EDTA preserved human blood and bone marrow aspirate specimens that have been collected and handled as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross - contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.

Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used in the amplification step become contaminated.

Work Areas

It is recommended that 3 dedicated areas within the laboratory be used when performing the Abbott RealTi*me* IDH2 assay. Physically separating the activities involved when performing PCR are measures taken to minimize the risk of contamination.

- The **Reagent Preparation Area** is dedicated to preparing reagents. All reagents used in the Reagent Preparation Area should remain in this dedicated area at all times. Pipettes, pipette tips, and vortex mixers used in the Reagent Preparation Area must remain in this area and not be moved to the other areas. Do not bring samples or amplification products into the Reagent Preparation Area.
- The **Sample Preparation Area** is dedicated to processing samples (specimens and controls) and to adding processed samples and controls to the Abbott 96 - Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortex mixers used in the Sample Preparation Area must remain in this area and not be moved to the Reagent Preparation and Amplification Areas. Do not bring amplification product into the Sample Preparation Area.
- The **Amplification Area** is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Reagent Preparation and Sample Preparation Areas.
- Work areas and instruments must be considered potential sources of contamination. Change gloves after contact with potential contaminant (such as DNase, specimens, eluates, and / or amplified products) or before handling unopened reagents, controls, or specimens. Refer to the Abbott *m*2000*rt* Operations Manual and the instructions in the **POST PROCESSING PROCEDURES** section for cleaning procedures.
- If the assay procedure is incorrectly performed or is interrupted at any point so that the timing of the steps exceeds the required timing, dispose of all commodities and single - use reagents that have been used in the assay procedure according to the instructions in the **POST PROCESSING PROCEDURES** section. For all completed, interrupted, or aborted Abbott *m*2000*rt* instrument runs, dispose of the Abbott 96 - Well Optical Reaction Plate in a sealed plastic bag according to the Abbott *m*2000*rt* Operations Manual. The gloves used to handle the plate should be disposed of in a waste container.
- Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous materials in accordance with local, state, and federal regulations.[16,17]
- All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

Note: Autoclaving the sealed reaction plate will not eliminate the amplified product and may contribute to the release of the amplified product by opening of the seal. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained before and after processing.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used. The pipette tips must be used only one time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*2000*rt* Operations Manual and the instructions in the **POST PROCESSING PROCEDURES** section.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of DNase contamination, cross-contamination between samples, and inhibition:

- Wear appropriate personal protective equipment at all times.
- Use powder - free gloves.
- Change gloves after contact with potential contaminants (specimens, eluates, and / or amplified product).
- Pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container.

STORAGE INSTRUCTIONS

Abbott RealTi*me* IDH2 Amplification Reagent Kit (List No. 08N82-090)

The Abbott RealTi*me* IDH2 Amplification Reagent Kit (List No. 08N82-090) must be stored at –25 to –15°C when not in use. Care must be taken to separate the Abbott RealTi*me* IDH2 Amplification Reagent Kit that is in use from direct contact with samples and controls.

Abbott RealTi*me* IDH2 Control Kit (List No. 08N82-080)

The Abbott RealTi*me* IDH2 Control Kit (List No. 08N82-080) must be stored at –25 to –15°C.

SHIPPING CONDITIONS

Component	Shipping Condition
Abbott RealTi*me* IDH2 Amplification Reagent Kit	Dry Ice
Abbott RealTi*me* IDH2 Control Kit	Dry Ice

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When control values are out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

If you receive reagents or controls that are in a condition contrary to label recommendation, or that are damaged, contact Abbott Molecular Customer Service.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Blood and Bone Marrow Aspirate Specimen Collection and Storage

Human blood (EDTA) and bone marrow aspirate (EDTA) specimens may be used with the Abbott RealTi*me* IDH2 assay. Follow the manufacturer's instructions for processing collection tubes. After collection, specimens may be stored:

- At 15 to 30°C for up to 48 hours
- At 2 to 8°C for up to 7 days
- At −20°C ± 5°C for longer term

Multiple freeze/thaw cycles should be minimized and should not exceed 3 freeze/thaw cycles. Document the number of freeze/thaws. Thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 24 hours.

Specimen Transport

Ship specimens with cold packs/boxes. The total time during transport should not exceed 48 hours. When shipment within 48 hours is not achievable, ship specimens on dry ice. Transport time on dry ice should not exceed 7 days provided that the dry ice is refilled during shipment and that the specimens remain frozen throughout transport. For domestic and international shipments, specimens should be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

INSTRUMENT PROCEDURE

The Abbott RealTi*me* IDH2 application specification file must be installed on the Abbott *m*2000*rt* instrument from the Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM prior to performing the assay. For detailed information on application specification file installation, refer to the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

ABBOTT REALTI*ME* IDH2 ASSAY PROCEDURE

Materials Provided

- Abbott RealTi*me* IDH2 Amplification Reagent Kit (List No. 08N82-090)

Materials Required But Not Provided

- Abbott RealTi*me* IDH2 Control Kit (List No. 08N82-080)

Reagent Preparation Area

- Calibrated precision pipettes capable of delivering 10 to 1000 µL
- 10 to 1000 µL PCR-grade aerosol barrier pipette tips for precision pipettes
- Vortex mixer

Sample Preparation Area

- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24)
- Magnetic rack (12-position) for 2.0 mL microtubes
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Abbott Splash-Free Support Base (List No. 9K31)
- Abbott Optical Adhesive Cover (List No. 04J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32)
- USP grade 190 to 200 proof (95 to 100%) ethanol. **Do not use ethanol that contains denaturants.**
- 2.0 mL screw-cap microtubes (Sarstedt Cat. No. 72.693.005 or equivalent)
- 2.0 mL screw-caps (Sarstedt Cat. No. 65.716.725 or equivalent)
- 50 mL polypropylene centrifuge tubes
- Graduated cylinder, 100 mL
- Serological pipettes
- Timer
- Calibrated precision pipettes capable of delivering 10 to 1000 µL
- 10 to 1000 µL PCR-grade aerosol barrier pipette tips for precision pipettes
- Microcentrifuge racks
- Vortex mixer
- Centrifuge capable of 1200 to 1500 *g* with rotor to accommodate Abbott 96-Well Optical Reaction Plate
- Heat blocks, 56 ± 3°C and 75 ± 5°C, suitable for 2.0 mL microtubes

Amplification Area

- Abbott *m*2000*rt* Instrument, List No. 09K15-01 (with System Software Version 8.0 or higher)
- Abbott RealTi*me* IDH2 *m*2000*rt* Application CD-ROM (List No. 08N82-001)
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 04J71-93)

Other Materials

- Lab coat
- Powder-free disposable gloves
- Protective eyewear
- Solid waste container
- Liquid waste container
- Sealable plastic bags

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples. The Abbott RealTi*me* IDH2 Amplification Reagent Kit is intended for use with the Abbott RealTi*me* IDH2 Control Kit and the Abbott *m*Sample Preparation System$_{DNA}$ kit for sample processing, and the Abbott *m*2000*rt* instrument for amplification and detection.
- For a detailed description of how to operate the Abbott *m*2000*rt* instrument, refer to the Abbott *m*2000*rt* Operations Manual, **Operating Instructions** section.
- Laboratory personnel must be trained to operate the Abbott *m*2000*rt* instrument. The operator must have thorough knowledge of the assay application run on the instrument and must follow good laboratory practices.
- Do not use kits or reagents beyond the expiration date.
- All kits should be stored at the proper temperature upon receipt. Refer to the **STORAGE INSTRUCTIONS** section for details. Do not use incorrectly stored reagents.
- Kit components from the same kit lot are intended to be used together. For example, do not use the Oligonucleotide Reagent from kit lot X with the DNA Polymerase Reagent from kit lot Y.
- In the Abbott RealTi*me* IDH2 Amplification Reagent Kit, the Oligonucleotide Reagent and Activation Reagent vials are intended for single preparation only and should be discarded after use.
- In the Abbott RealTi*me* IDH2 Control Kit, each Negative Control and Positive Control vial is intended for single use only and should be discarded after use.
- The Amplification Reagent Kit and Control Kit can be thawed and refrozen up to 3 times before use.
- The use of the Abbott RealTi*me* IDH2 Positive Control and Negative Control are integral to the performance of the Abbott RealTi*me* IDH2 assay. Abbott RealTi*me* IDH2 controls must be processed alongside the specimens to be tested. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.
- Use only USP Grade 190 to 200 proof (95 to 100%) ethanol to prepare the *m*Wash 2$_{DNA}$ Buffer. **Do not use ethanol that contains denaturants.**
- Use aerosol-barrier pipette tips. The use of extended aerosol-barrier pipette tips is recommended to prevent contamination.
- Ensure that each sample eluate is transferred into the correct locations of the Abbott 96-Well Optical Reaction Plate designated by the test order. Refer to the **Abbott *m*2000*rt* Initiation and Test Order Creation** section of this package insert for details.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens, reagents, and controls by using a detergent solution followed by a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectants. Refer to **Safety Precautions** for additional instructions.

ASSAY PROTOCOL

The Abbott RealTi*me* IDH2 assay protocol includes the following steps:

A. Manual preparation (ie, DNA extraction) of samples (specimens and controls) using the Abbott *m*Sample Preparation System$_{DNA}$ Kit.

B. PCR assay setup using the sample eluates and the Abbott RealTi*me* IDH2 Amplification Reagent Kit.

C. Amplification/detection on the Abbott *m*2000*rt* instrument.

Refer to the **WARNINGS AND PRECAUTIONS** section of this package insert for instructions before preparing samples. At least one Positive Control and one Negative Control must be included in each run.

Note: Each Abbott *m*Sample Preparation System$_{DNA}$ Kit contains 4 sets of reagents. Each set of reagents can support preparation (ie, DNA extraction) of up to 24 samples (patient specimens and/or assay controls). Discard any reagents remaining after 24 preparations.

Note: Per magnetic rack, a maximum of 12 samples (patient specimens and/or assay controls) can undergo DNA extraction. It is not recommended to perform DNA extraction in batch sizes that exceed 12 samples.

Note: Each Abbott RealTi*me* IDH2 Amplification Reagent Kit supports testing of up to 24 samples (patient specimens and/or assay controls).

Reagent Preparation Area

Thawing of Amplification Reagents

New or previously prepared master mixes may be used (see **Preparation of Amplification Master Mixes**).

1. If a new master mix is needed, thaw the Oligonucleotide Reagents and Activation Reagent at 15 to 30˚C or at 2 to 8˚C.
   - Once thawed, if the amplification reagents are not being used immediately, they can be stored at 2 to 8˚C for up to 24 hours until required for preparation of the amplification master mixes.

   OR
   - If previously prepared frozen master mixes are used, thaw the master mixes at 15 to 30˚C for up to 30 minutes prior to PCR setup. Frozen master mixes should not undergo more than 5 freeze/thaw cycles.

Sample Preparation Area

Thawing of Assay Controls

2. Thaw assay controls at 15 to 30˚C or at 2 to 8˚C. One Positive Control and one Negative Control are required in each DNA extraction run; see the **QUALITY CONTROL PROCEDURES** section of the package insert.
   - Once thawed, if the assay controls are not being processed immediately, they can be stored at 2 to 8˚C for up to 24 hours prior to DNA extraction.

Preparation of Archived Specimens

3. Thaw blood or bone marrow aspirate specimens at 15 to 30˚C or at 2 to 8˚C.
   - Once thawed, if blood or bone marrow aspirate specimens are not being processed immediately, they can be stored at 2 to 8˚C for up to 24 hours.

Preparation of Heat Blocks

4. Turn on the temperature controlled dry heating blocks:

   a. Set one heat block at 56±3˚C

   b. Set one heat block at 75±5˚C

   If only one heat block is available then set to 56±3˚C and equilibrate to 75±5˚C after step 28.

   Note: Check the temperature of the heating block(s). Do not proceed until the heating block(s) are at the correct temperature.

   WARNING: To avoid personal injury, follow the manufacturer's instructions for heat blocks. To avoid burns, turn off the power and allow the heat blocks to cool to 35˚C or below before handling.

Preparation of DNA Extraction Reagents

5. Remove one set of Abbott *m*Sample Preparation System$_{DNA}$ reagent bottles from the kit, and gently invert each bottle except *m*Microparticles$_{DNA}$ to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents without allowing the crystals to dissolve.

Note: Each set of Abbott *m*Sample Preparation System$_{DNA}$ reagents (labeled as "No Reuse") may be used for multiple DNA extraction runs, depending on the number of samples processed per run.

6. Prepare the *m*Wash 2$_{DNA}$ Buffer working solution by adding 70 mL of 190 to 200 proof (95 to 100%) ethanol to the *m*Wash 2$_{DNA}$ bottle. Cap the bottle and gently invert 5 to 10 times to mix. Mark the bottle to indicate ethanol has been added, and assign an expiration date of 2 months from the date of preparation. Store at 15 to 30˚C.

DNA Extraction

Lysis

7. Label a 2.0 mL Sarstedt tube or equivalent for each patient specimen and assay control.
   - One IDH2 Negative Control and one IDH2 Positive Control must be processed alongside the patient specimens to be tested.
8. Resuspend *m*Microparticles$_{DNA}$ by vortexing or vigorously shaking until particles are in suspension and settled particles are no longer seen on the bottom of the bottle. After the particles are resuspended, add 300 µL of *m*Microparticles$_{DNA}$ to each tube.
9. Add 1.3 mL of *m*Lysis$_{DNA}$ to each 2.0 mL tube.
10. Prepare patient specimens and control.
    - Gently invert each patient specimen a minimum of 10 times to ensure homogeneity.
    - Vortex each assay control 3 times for 2 to 3 seconds before use. After vortexing, tap each vial on the bench to bring the liquid to the bottom of the vial.
11. Add patient specimen or controls to the appropriate tubes.
    - For controls add 200 µL.
    - For blood specimens add 200 µL.
    - For bone marrow aspirate specimens add 100 µL.

    The Positive Control and Negative Control vials are intended for single use only; remaining reagents after first use should be discarded.
12. Invert each tube three times and vortex for 30 seconds.
13. Place the tubes in the 56˚C heating block for 7±1 minutes.
14. Remove the tubes from the heating block and vortex for 5 seconds.
15. Place the tubes in the 56˚C heating block for 8±1 minutes.

Wash

16. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
17. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

    Do not disturb or aspirate the captured magnetic particles.
18. Place the tubes in a microcentrifuge rack and for each tube:
    - Add 900 µL of *m*Lysis$_{DNA}$.
    - Attach a new cap.
    - Vortex for 5 seconds.
19. Place the tubes in the 56˚C heating block for 3 ±1 minutes.
20. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
21. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

    Do not disturb or aspirate the captured magnetic particles.
22. Place the tubes in a microcentrifuge rack and for each tube:
    - Add 900 µL of *m*Wash 1$_{DNA}$.
    - Attach a new cap.
    - Vortex for 5 seconds.
23. Place the tubes in the 56˚C heating block for 1 to 2 minutes.
24. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.

25. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

    Do not disturb or aspirate the captured magnetic particles.

26. Place the tubes in a microcentrifuge rack and for each tube:
    - Add 900 µL of *m*Wash 1_{DNA}.
    - Attach a new cap.
    - Vortex for 5 seconds.
27. Place the tubes in the 56˚C heating block for 1 to 2 minutes.
28. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
29. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

    Do not disturb or aspirate the captured magnetic particles.

30. Place the tubes in a microcentrifuge rack and for each tube:
    - Add 900 µL of *m*Wash 2_{DNA}.
    - Attach a new cap.
    - Vortex for 5 seconds.
31. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
32. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

    Do not disturb or aspirate the captured magnetic particles.

33. Place the tubes in a microcentrifuge rack and for each tube:
    - Add 900 µL of *m*Wash 2_{DNA}.
    - Attach a new cap.
    - Vortex for 5 seconds.
34. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
35. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

    Do not disturb or aspirate the captured magnetic particles.

36. Place the tubes in the 75˚C heating block for 13±1 minutes.

    Note: The tubes should not be capped.

DNA Elution

37. Place the tubes in a microcentrifuge rack and for each tube:
    - Add 500 µL of *m*Elution $Buffer_{DNA}$.
    - Attach a new cap.
    - Vortex for 5 seconds.
38. Place the tubes in the 75˚C heating block for 6±1 minutes.
    - During the incubation, label new 2.0 mL tubes for the DNA eluates.
39. Vortex for 5 seconds.
40. Return the tubes in the 75˚C heating block for 6±1 minutes.
41. Vortex for 5 seconds.
42. Return the tubes in the 75˚C heating block for 6±1 minutes.
43. Vortex for 5 seconds.
44. Return the tubes in the 75˚C heating block for 6±1 minutes.
45. Vortex for 5 seconds.
46. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
47. With the tube in the magnetic rack, use a fresh, sterile 1000 µL aerosol barrier pipette tip for each sample to carefully transfer the eluted sample from each tube into a new, labeled 2.0 mL tube.

    Do not disturb or aspirate the captured magnetic particles.

    DNA eluates for specimens or controls can be stored at:
    - -25 to -15˚C or colder for up to 2 months, and should not undergo more than 5 freeze/thaw cycles;
    - 15 to 30˚C for 24 hours;
    - 2 to 8˚C for 14 days;

    Frozen DNA eluates can be thawed at 15 to 30˚C or at 2 to 8˚C prior to PCR setup. Storage of DNA eluates must not exceed a cumulative total of 24 hours at 15 to 30˚C or 14 days at 2 to 8˚C prior to PCR setup.

Amplification Area

Abbott *m*2000rt Initiation and Test Order Creation

48. Switch on and initialize the Abbott *m*2000*rt* instrument.
49. Create an Abbott *m*2000*rt* test order. Refer to the **Operating Instructions** section of the Abbott *m*2000*rt* Operations Manual. From the Protocol screen, select the appropriate application specification file corresponding to the Abbott RealTi*me* IDH2 assay.
50. A 12-sample (2 controls and 10 specimens) setup for the Abbott 96-well reaction plate is shown in Figure 1 below.

Figure 1: Example of a 12 Sample Setup for Abbott 96-Well Optical Reaction Plate

Example Plate Setup

	1	2	3	4	5	6	7	8
A	NEG	NEG	NEG	NEG	#7	#7	#7	#7
B	POS	POS	POS	POS	#8	#8	#8	#8
C	#1	#1	#1	#1	#9	#9	#9	#9
D	#2	#2	#2	#2	#10	#10	#10	#10
E	#3	#3	#3	#3				
F	#4	#4	#4	#4				
G	#5	#5	#5	#5				
H	#6	#6	#6	#6				

Oligo 1 master mix; Oligo 2 master mix; Oligo 3 master mix; Oligo 4 master mix; Oligo 1 master mix; Oligo 2 master mix; Oligo 3 master mix; Oligo 4 master mix

Figure 1 represents a partial image of the Abbott 96-Well Optical Reaction Plate.

Each control or sample must be placed in 4 adjacent wells, as indicated in Figure 1 and as described below:

- Oligo 1 master mix may only be added to columns 1, 5, and 9.
- Oligo 2 master mix may only be added to columns 2, 6, and 10.
- Oligo 3 master mix may only be added to columns 3, 7, and 11.
- Oligo 4 master mix may only be added to columns 4, 8 and 12.

Negative control (NEG), Positive Control (POS), and specimens may be placed anywhere on the plate, following the 4 adjacent well requirement.

Note: Remove gloves before returning to the Reagent Preparation Area.

Reagent Preparation Area

Preparation of the Amplification Master Mix

A. If using previously prepared Oligonucleotide-specific master mixes, proceed to PCR Setup.

B. If combining multiple vials of Oligonucleotide-specific master mixes:
    - Only combine Oligonucleotide-specific master mixes from the same Amplification Reagent Kit lot.
    - Only combine Oligonucleotide-specific master mixes (eg, only combine Oligonucleotide 1 master mix with Oligonucleotide 1 master mix).
    - Only combine a volume of Oligonucleotide-specific master mix sufficient for current testing. Once combined, remaining volume of combined Oligonucleotide-specific master mixes should be discarded.
    - Combine Oligonucleotide-specific master mixes in a 2.0 mL microcentrifuge tube or 5 mL tube. The required volume of each Oligonucleotide-specific master mix is 45 µL × (total number of samples and controls + 1). Mix by gently pipetting up and down 5 times. Ensure that no foam or bubbles are created. Label the tube with the appropriate master mix designation (eg, Oligonucleotide 1 master mix).

C. If preparing new Oligonucleotide-specific master mixes:

1. Prior to opening the amplification reagents, tap each vial in an upright position on the bench to bring the liquid to the bottom.
2. Gently mix contents of Oligonucleotide Reagent and Activation Reagent vials by pipetting up and down 5 times prior to use.

   Note: To prepare master mixes, use a calibrated precision pipettor designated for master mix reagent use only.

   - Prepare Oligo 1 master mix by adding the following to a new DNA Polymerase vial:

     a. 870 µL of Oligonucleotide Reagent 1.

     b. 275 µL of Activation Reagent.

     Vortex vial 3 times for 2 to 3 seconds. Minimize the creation of foam or bubbles. Label as "Oligo 1 master mix."
   - Prepare Oligo 2 master mix by adding the following to a new DNA Polymerase vial:

     a. 870 µL of Oligonucleotide Reagent 2.

     b. 275 µL of Activation Reagent.

     Vortex vial 3 times for 2 to 3 seconds. Minimize the creation of foam or bubbles. Label as "Oligo 2 master mix."
   - Prepare Oligo 3 master mix by adding the following to a new DNA Polymerase vial:

     a. 870 µL of Oligonucleotide Reagent 3.

     b. 275 µL of Activation Reagent.

     Vortex vial 3 times for 2 to 3 seconds. Minimize the creation of foam or bubbles. Label as "Oligo 3 master mix."
   - Prepare Oligo 4 master mix by adding the following to a new DNA Polymerase vial:

     a. 870 µL of Oligonucleotide Reagent 4.

     b. 275 µL of Activation Reagent.

     Vortex vial 3 times for 2 to 3 seconds. Minimize the creation of foam or bubbles. Label as "Oligo 4 master mix."

     Note: Oligonucleotide Reagent and Activation Reagent vials are intended for single preparation only. Remaining reagents after first preparation should be discarded.
     Note: The Abbott *m2000rt* protocol (PCR Run Initiation, step 62) must be initiated within 60 minutes after preparation of the amplification master mixes.
     Note: Remove gloves before returning to the Sample Preparation Area.

Sample Preparation Area

PCR Setup

51. Place an Abbott 96-Well Optical Reaction Plate onto an Abbott Splash-Free Support Base. DO NOT touch the surface or bottom of the plate.
52. Prior to use, mix the amplification master mix by gently pipetting up and down 5 times. Ensure that no foam or bubbles are created.
53. Pipette 45 µL of the master mix into the required number of wells of the Abbott 96-Well Optical Reaction Plate according to the test order.
54. Store the remainder of prepared master mixes (optional):
    - Store the remaining master mixes tightly capped and protected from light for up to 7 days at 2 to 8˚C or 60 days at −25 to −15˚C.
    - Frozen master mixes cannot undergo more than 5 freeze/thaws.
55. Visually verify that 45 µL of master mix have been dispensed into each designated well.
56. Prior to use, vortex each sample DNA eluate tube for 10 seconds. After vortexing, tap each tube on the bench to bring the liquid to the bottom of the tube.
57. Pipette 15 µL of DNA eluate into each well designated for a sample according to the test order. During the transfer of each eluate, mix the reaction by pipetting up and down 3 to 5 times. Change pipette tips between wells.
58. Visually verify that 60 µL total have been dispensed into each designated well.
59. Seal the Abbott 96-Well Optical Reaction Plate with the Abbott Optical Adhesive Cover according to the instructions in the Abbott *m2000rt* Operations Manual.
60. Ensure that all material is at the bottom of each well. If needed, centrifuge the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 1200 to 1500 *g* for 1 minute.

Amplification Area

PCR Run Initiation

61. Transfer the Abbott 96-Well Optical Reaction Plate from the Abbott Splash-Free Support Base to the Abbott *m2000rt* instrument.
62. Select the Abbott *m2000rt* test order created in **Abbott m2000rt Initiation and Test Order Creation**, Step 49. Initiate the run, as described in the Abbott *m2000rt* Operations Manual, Operating Instructions section.
63. At the completion of the run, assay results are reported on the Abbott *m2000rt* instrument. Refer to the **INTERPRETATION OF RESULTS** section of the package insert for further details.

POST PROCESSING PROCEDURES

1. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual, along with the gloves used to handle the plate.
2. Clean the Abbott Splash-Free Support Base before next use, according to Abbott *m2000rt* Operations Manual.
3. Decontaminate and dispose of all specimens, controls, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.
4. Remove and discard all disposables, as well as liquid and solid waste, in accordance with local, state, and federal regulations.
5. At the end of each run, clear and clean all work areas. Decontaminate work area according to laboratory guidelines.

QUALITY CONTROL PROCEDURES

Abbott *m2000rt* Optical Calibration

Refer to the **Calibration Procedures** section in the Abbott *m2000rt* Operations Manual for a detailed description of how to perform an Abbott *m2000rt* Optical Calibration. Optical calibration of the Abbott *m2000rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*me* IDH2 assay. The following Abbott *m2000rt* Optical Calibration Plates are used to calibrate the Abbott *m2000rt* instrument for the Abbott RealTi*me* IDH2 assay:

- FAM™ Plate (Carboxyfluorescein)
- VIC™ Plate (Proprietary dye)
- NED™ Plate (Proprietary dye)
- ROX™ Plate (Carboxy-X-rhodamine)
- Cy5 Plate (Cyanine)

Positive and Negative Controls

A Positive Control and a Negative Control are required in each run to verify that the sample processing, the amplification, and the detection steps are performed correctly. The Abbott RealTi*me* IDH2 controls are processed alongside the specimens prior to running the amplification portion of the assay.

The Positive Control is formulated with DNA containing IDH2 mutation and IC sequences. R140Q, R140W, R172K, and R172W mutations should be detected for the Positive Control. In addition, the IC should be detected in all 4 reactions for the Positive Control.

The Negative Control is formulated with DNA containing the IC sequence. Only the IC should be detected in all 4 reactions for the Negative Control.

IDH2 mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) should not be detected in the Negative Control. IDH2 mutations detected in the Negative Control are indicative of contamination from other samples or amplified product introduced during sample processing or during preparation of the Abbott 96-Well Optical Reaction Plate. To remove contamination, clean the working area, the equipment, and the Abbott *m2000rt* instrument according to the Abbott *m2000rt* Operations Manual and the instructions in the **POST PROCESSING PROCEDURES** section. Following cleaning, repeat sample processing for specimens and controls.

IC results for the Negative Control and the Positive Control that are outside the validity limit may indicate the occurrence of inhibition during DNA extraction or during the amplification reaction steps of the assay. Repeat the processing for specimens (starting from specimen preparation) and controls (starting from DNA extraction).

If the result for the Negative Control or the Positive Control is out of range, the run is invalid and a flag is displayed for each specimen. Refer to "Assay Specific Error Codes" and "Repeating Invalid Runs or Invalid Samples."

Monitoring the Laboratory for the Presence of Contamination

It is recommended that this be done when contamination is suspected (eg, by amplification product, patient specimen, or Positive Control). Contamination may be indicated by a Negative Control failure. It is very important to test all areas that may have been exposed to processed specimens and controls, and/or amplification product. This includes routinely handled objects such as pipettes, function keys for the Abbott *m*2000*rt*, magnetic racks, temperature blocks, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.6 mL molecular biology grade water to a 1.7 mL DNase-free microcentrifuge tube for each laboratory surface area to be monitored.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the molecular biology grade water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in molecular biology grade water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. For each additional area to be monitored, repeat steps 2 through 4.
6. Test the samples according to the **ASSAY PROTOCOL** section of this package insert using a sample input volume of 200 µL for each swab sample.
7. Contamination is indicated by the presence of a positive CN value for R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, or R172W in the swab sample(s). If contamination is not present, CN values for R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S or R172W will be -1 in the swab samples.

   Note: The reported result and interpretation are not used to determine contamination because the IC signal may not be detected in the swab samples (ie, water).
8. If an IDH2 mutation is detected on equipment, follow the cleaning and decontaminating guidelines given in the equipment operations manual. If IDH2 is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water. Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.
9. Repeat testing of the contaminated area by following steps 1 through 6.
10. If the presence of contamination is detected again, repeat steps 8 and 9 until amplification of IDH2 mutation(s) is no longer detected.

INTERPRETATION OF RESULTS

The Abbott *m*2000*rt* instrument automatically reports the results on the *m*2000*rt* workstation. Examples of assay results and interpretations are provided in **Table 3**.

The Abbott RealTi*me* IDH2 assay controls are used to establish run validity for the Abbott RealTi*me* IDH2 assay. If an error code is generated for one or both assay controls, the run is invalid, and a "–QC" flag and/or "+QC" flag are displayed next to all specimen results.

Table 3. Examples of Assay Results

Sample ID	Result	Interpretation
Sample 1	R140Q[a]	Mutation Detected
Sample 2	Not Detected	Not Detected

[a] The displayed result will correspond to the mutation detected by the assay.

Results from all 4 PCR wells are evaluated for each specimen. If a mutation is detected, an interpretation of "Mutation Detected" will be displayed and the result field will display the identity of the mutation. If no mutations are detected, an interpretation of "Not Detected" will be displayed and the result field will display "Not Detected."

Assay Specific Error Codes

Error codes specific to the Abbott RealTi*me* IDH2 assay are listed in **Appendix 1** and **Appendix 2**. For each error code, probable causes and corrective actions specific to the Abbott RealTi*me* IDH2 are also listed. Information for additional error codes is presented in the Abbott *m*2000*rt* Operations Manual, Version 8.0 or higher, **Troubleshooting and Diagnostics** section.

Repeating Invalid Runs or Invalid Samples

An invalid run in which an error code was generated for one or both assay controls should be repeated following the guidelines below.

- If the invalid run was due to an assignable error in plate setup or test order creation, repeat the run starting with **Abbott *m*2000*rt* Initiation and Test Order Creation**, by using the remaining eluates (controls and specimen(s)).
- If there was no assignable error, all of the controls and specimens must be reprocessed, beginning with sample preparation.

If an error code was generated for a patient specimen, the specimen should be retested following the guidelines below.

- If the error code was due to an assignable error in plate setup or test order creation, repeat the run starting with **Abbott *m*2000*rt* Initiation and Test Order Creation**, by using the remaining eluates (controls and specimen(s)).
- If there was no assignable error, the specimen that received the error code and controls must be reprocessed, beginning with sample preparation.

LIMITATIONS OF THE ASSAY

- **FOR IN VITRO DIAGNOSTIC USE.**
- Abbott RealTi*me* IDH2 is for use with human blood (EDTA) and bone marrow aspirate (EDTA) specimens only.
- Optimal performance of this test requires appropriate specimen collection, handling, preparation, and storage (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Use of the Abbott RealTi*me* IDH2 assay is limited to personnel who have been trained in the procedures of molecular diagnostic assays, the Abbott *m*2000*rt* instrument, and the manual sample preparation method for Abbott RealTi*me* IDH2.
- A "Not Detected" result does not preclude the presence of IDH2 mutations in the specimen. Assay results may be affected by inadequate specimen integrity, mutation content in the sample, and amount of amplifiable DNA.
- The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive control or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.
- Abbott RealTi*me* IDH2 is designed to detect IDH2 R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W mutations. Specimens with results reported as "Not Detected" may contain mutations that are not targeted by the assay.
- Analytical studies indicate that at high mutation percentage and high DNA input, the R140W probe can cross react with the R140G mutation. In this case, the software reports the mutation which is present at the highest level. All IDH2 mutations were correctly called in the analytical studies.
- Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the IDH2 Oligonucleotide Reagents from Amplification kit lot X with the DNA Polymerase Reagent from Amplification kit lot Y.

SPECIFIC PERFORMANCE CHARACTERISTICS

Bone marrow aspirate and blood were tested in all key analytical studies. For the most prevalent IDH2 mutations, AML clinical specimens were used, when possible; otherwise cell lines with IDH2 mutations were used. Ten separate cell lines were used in the analytical studies, each cell line contained a single IDH2 mutation and represented all 10 IDH2 mutations that can be detected by Abbott RealTi*me* IDH2 including R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S (cell lines for both R172St and R172Sc), and R172W. For the Repeatability, Lot-to-Lot Reproducibility, Extracted Clinical Specimen DNA Stability, Potentially Interfering Substances, Potentially Interfering Drugs, Potentially Interfering Microorganisms, and Analytical Carryover studies, cell lines were spiked into blood or bone marrow to target specific mutation percentages. For the Analytical Sensitivity–Limit of Detection, Characterization of IDH2 Mutation Detection at DNA Input Levels Across and Below Assay Range, and Activated Master Mix Use and Storage and Assembled Plate Stability studies, DNA eluates generated from blood or bone marrow spiked with cell lines were diluted to target specific mutation percentages and gDNA inputs. Functional equivalence studies were used to demonstrate that contrived specimens created using cell lines had comparable performance to clinical specimens in terms of limit of detection and precision.

Abbott RealTi*me* IDH2 is designed such that quantitation of DNA prior to PCR amplification is not required. A fixed amount of eluate (15 µL) from sample extraction is used in each PCR reaction. In order to evaluate the typical DNA yield from AML specimens, DNA was extracted from 124 AML clinical specimens (74 bone marrow aspirate specimens and 50 blood specimens) with the Abbott *m*Sample Preparation System$_{DNA}$ Kit. The DNA yield, as measured by the IC CN value, was similar for bone marrow and blood. The gDNA input range observed for bone marrow aspirate was 14 ng to 1,998 ng per 15 µL of eluate, which corresponds to an IC CN range of 15.01 CN to 22.00 CN. The gDNA input range observed for whole blood was 22 ng to 1,182 ng per 15 µL of eluate, which corresponds to an IC CN range of 15.77 CN to 21.50 CN. Abbott RealTi*me* IDH2 assay performance was verified across a range of 10 ng to 2,000 ng gDNA input per PCR reaction.

ANALYTICAL SENSITIVITY

Limit of Detection

Analytical sensitivity was assessed using eluates generated from blood and bone marrow samples. The IDH2 percent mutation ranged from 0.25% to 10% for 10 ng gDNA input and from 0.13% to 10% mutation for 200 ng gDNA input. A 28 member panel was generated for each of the 10 SNVs targeted by the assay. IDH2 R140Q, R172K, and R140L mutation positive panel members were prepared from blood and bone marrow aspirate AML clinical specimen eluates. IDH2 R140G, R140W, R172M, R172S (AGT and AGC), R172W, and R172G mutation positive panel members were prepared from eluates of IDH2 mutation positive cell lines spiked into blood or bone marrow. Each panel member was tested with 2 Abbott RealTi*me* IDH2 Amplification Reagent Kit lots with 5 runs per lot over a minimum of 5 days and 4 replicates per run for a total of 40 replicates per panel member per mutation and 400 replicates for the 10 mutations combined.

The analysis demonstrated a detection rate of 99.8% (399/400) or greater at mutation levels of 2% and higher for all IDH2 mutations combined. The analysis demonstrated a detection rate of 93.5% (374/400) or greater at mutation levels of 1% and higher for all IDH2 mutations combined. Refer to **Table 4** for results.

Table 4: Limit of Detection Study Results for all IDH2 Mutations[a] Combined

gDNA Input per Reaction	Sample Type	Mutation %	Detection Ratio	Detection Rate
200 ng	Blood	10%	400/400	100%
200 ng	Blood	5%	400/400	100%
200 ng	Blood	2%	400/400	100%
200 ng	Blood	1%	400/400	100%
200 ng	Blood	0.5%	400/400	100%
200 ng	Blood	0.25%	384/400	96%
200 ng	Blood	0.13%	283/400	70.8%
200 ng	Bone Marrow	10%	400/400	100%
200 ng	Bone Marrow	5%	400/400	100%
200 ng	Bone Marrow	2%	400/400	100%
200 ng	Bone Marrow	1%	400/400	100%
200 ng	Bone Marrow	0.5%	400/400	100%
200 ng	Bone Marrow	0.25%	400/400	100%
200 ng	Bone Marrow	0.13%	301/400	75.3%
10 ng	Blood	10%	400/400	100%
10 ng	Blood	5%	400/400	100%
10 ng	Blood	4%	400/400	100%
10 ng	Blood	2%	399/400	99.8%
10 ng	Blood	1%	374/400	93.5%
10 ng	Blood	0.5%	271/400	67.8%
10 ng	Blood	0.25%	111/400	27.8%
10 ng	Bone Marrow	10%	400/400	100%
10 ng	Bone Marrow	5%	400/400	100%
10 ng	Bone Marrow	4%	400/400	100%
10 ng	Bone Marrow	2%	400/400	100%
10 ng	Bone Marrow	1%	389/400	97.3%
10 ng	Bone Marrow	0.5%	265/400	66.3%
10 ng	Bone Marrow	0.25%	109/400	27.3%

[a] Includes R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172St, R172Sc, and R172W, 40 replicates per mutation.

Characterization of IDH2 Mutation Detection Across a Low to High Range of DNA Input Levels

This study evaluated the detection of IDH2 mutations targeted by the assay at several gDNA inputs across a range of 1 ng to 2,000 ng. For each IDH2 SNV, the following gDNA inputs were tested at 2% mutation level in both blood and bone marrow aspirate: approximately 1 ng, 50 ng, 600 ng, 1,000 ng, and 2,000 ng. A total of 100 panel members (10 per mutation) were each tested in triplicate. Three replicates per panel member per mutation were tested for a total of 30 replicates for the 10 mutations combined.

All panel members reported "Mutation Detected" at a 100% (30/30) detection rate when total gDNA input levels were 50 ng, 600 ng, 1,000 ng, and 2,000 ng per reaction. When the gDNA input level was 1 ng per reaction, an overall detection rate of 13% (4/30) was observed due to IC cycle number being nonreactive or greater than the maximum for non-detected replicates, as expected for the low input level. The valid replicates for the 1 ng per reaction input level reported "Mutation Detected" at a 100% detection rate. Refer to **Table 5** for results.

The analysis demonstrated a detection rate of 100% at mutation levels of approximately 2% when the total gDNA input was approximately 50 ng, 600 ng, 1,000 ng, and 2,000 ng per reaction for all mutations tested.

Table 5. Detection of 2% Mutation Level for a Range of DNA Inputs for all IDH2 Mutations[a] Combined

Sample Type	gDNA Input per Reaction (ng)	Number Valid Replicates	Number of Replicates Mutation Detected	Valid Replicate Detection Rate (%)	Total Number of Replicates	Overall Detection Rate (%)
Blood	1	4	4	100	30	13
Blood	50	30	30	100	30	100
Blood	600	30	30	100	30	100
Blood	1,000	30	30	100	30	100
Blood	2,000	30	30	100	30	100
Bone Marrow	1	4	4	100	30	13
Bone Marrow	50	30	30	100	30	100
Bone Marrow	600	30	30	100	30	100
Bone Marrow	1,000	30	30	100	30	100
Bone Marrow	2,000	30	30	100	30	100

[a] Includes R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172St, R172Sc, and R172W, 3 replicates per mutation

Limit of Blank

The Limit of Blank (LoB) was verified by testing 10 normal blood specimens, 10 normal bone marrow aspirate specimens, and 2 blank samples (water). Each specimen or sample was tested in replicates of 4, yielding a total of 88 replicates (40 replicates for whole blood specimens, 40 replicates for bone marrow aspirate specimens, and 8 replicates for blank samples). The overall rate of correct sample interpretation was 100% (88/88) which demonstrated an acceptable rate of true negativity.

ANALYTICAL SPECIFICITY

Exclusivity

This study evaluated the rate of correct sample interpretation for each Abbott RealTi*me* IDH2 target using a panel representing IDH2 SNVs targeted by the assay, IDH2 SNVs not targeted by the assay, and wild type genomic DNA. Panel members were prepared such that each PCR reaction contained a total input of approximately 200 ng of wild type human placental (HP) DNA, or wild type HP DNA plus plasmid DNA resulting in a percent mutation of approximately 25% to 50% in 200 ng of total DNA. Panel Members included wild type HP DNA (R140, R172) and wild type HP DNA plus one of 12 DNA plasmids coding for each of the 10 IDH2 SNVs targeted by the assay (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172Sc, R172St, R172W) or 2 IDH2 SNVs not targeted by the assay (R140P, R172T). The prepared panel members were directly tested using the Abbott RealTi*me* IDH2 Amplification Reagent Kit. Forty replicates per panel member were tested.

The wild type HP DNA panel member reported "Not Detected" with a 100% (40/40) rate of correct sample interpretation. Ten panel members targeted by the assay each reported "Mutation Detected" with a 100% (40/40) rate of correct sample interpretation, which included the correct identity of the IDH2 mutation. Two panel members not targeted by the assay (R140P mutation, and R172T mutation) reported "Not Detected" with a 100% (40/40) rate of correct sample interpretation.

In this study, R140W probe cross reactivity was observed for the panel member containing the R140G mutation. When two mutations are detected, the software calls the mutation which is present at the highest level. As shown in this study the R140G mutation was called correctly for all 40 panel member replicates, thus a 100% rate of correct sample interpretation was demonstrated.

The Abbott RealTi*me* IDH2 assay demonstrated a 100% (40/40) correct sample interpretation rate using a panel representing IDH2 SNVs targeted by the assay, IDH2 SNVs not targeted by the assay, and wild type genomic DNA.

Potentially Interfering Substances

Potentially interfering substances that may be found in clinical specimens were added to IDH2 mutation negative, IDH2 R140Q mutation positive, and IDH2 R172K mutation positive blood and bone marrow aspirate specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each test condition and each sample were evaluated for the presence of potential interference.

Test conditions included control diluents (one for bilirubin and one for all other substances) or 6 potentially interfering substances at study specified concentrations (hemoglobin, albumin, bilirubin, triglycerides, R(-)-2-hydroxyglutarate (2-HG), and EDTA). The analysis demonstrated the percent agreement was 100% (3/3) for each sample at each test condition.

No interference of the Abbott RealTi*me* IDH2 assay was observed in the presence of the potentially interfering substances for all IDH2 mutation positive and negative samples for either blood or bone marrow aspirate specimens.

Potentially Interfering Drugs

Potentially interfering drugs that may be found in clinical specimens were added to IDH2 mutation negative, IDH2 R140Q mutation positive, and IDH2 R172K mutation positive blood and bone marrow aspirate specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each test condition (control or Drug Pool 1 to 4) for each specimen was evaluated. The control condition tested specimens with no potentially interfering drugs added, whereas Drug Pool 1 to 4 tested specimens with added pooled drug combinations as described below:

Abbott RealTi*me* IDH2 Potentially Interfering Drug Pools

Pool 1	Pool 2	Pool 3	Pool 4
Vancomycin Hydrochloride	Levofloxacin	Linezolid	Meropenem
Busulfan	Acyclovir	Arsenic Trioxide	Azacytidine
Decitabine	Cyclophosphamide	Cytarabine	Daunorubicin Hydrochloride
Hydroxyurea	Doxorubicin Hydrochloride	Etoposide	Fludara
Mitoxantrone Hydrochloride	Idarubicin Hydrochloride	Lomustine	Melphalan
Everolimus	Vincristine Sulfate	Erythropoietin	Filgrastim
Dexamethasone	Mercaptopurine	Mycophenolate Mofetil	Tacrolimus
	Prednisone	AG-221	AG-120

Each drug was tested at three times the peak serum concentration (C_{MAX}), recommended dose as listed in the drug package inserts, or recommended concentration per Clinical and Laboratory Standards Institute EP7-A2.[18] The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition.

No interference of the Abbott RealTi*me* IDH2 assay was observed in the presence of the potential interfering drugs for all IDH2 mutation positive and negative samples for either blood or bone marrow aspirate specimens.

Potentially Interfering Microorganisms

Potentially interfering microorganisms were added to IDH2 mutation negative, IDH2 R140Q mutation positive, and IDH2 R172K mutation positive blood and bone marrow aspirate specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each of 5 test conditions (control and Microbe Pools 1 to 4) were evaluated. The microbe pools were as follows:

Abbott RealTi*me* IDH2 Potentially Interfering Microbe Pools

Pool 1	Pool 2	Pool 3	Pool 4
Staphylococcus aureus (MRSA; COL)	*Streptococcus agalactiae*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
Staphylococcus aureus (CoNS)	*Streptococcus pneumoniae*	*Enterobacter cloacae*	*Acinetobacter baumannii*
Staphylococcus epidermidis	*Klebsiella pneumoniae*	*Aspergillus terreus*	varicella-zoster virus
Escherichia coli	*Candida albicans*	influenza A H3	respiratory syncytial virus type A
Serratia marcescens	herpes simplex virus type 1 MacIntyre		respiratory syncytial virus type B
cytomegalovirus	herpes simplex virus type 2 MS		

Potentially interfering microorganisms were chosen based on clinical presentation in AML patients.[19] The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition.

No interference or cross reactivity of the Abbott RealTi*me* IDH2 assay was observed in the presence of the potentially interfering microorganisms for all IDH2 mutation positive and negative samples tested with either blood or bone marrow aspirate specimens.

PRECISION

Within-Laboratory Repeatability

The within-laboratory repeatability was evaluated by two operators using a 12-member panel. The panel contained IDH2 mutation negative blood and bone marrow aspirate specimens as well as blood and bone marrow aspirate specimens spiked with R140Q, R140W, R172K, or R172W positive cell lines targeting a 6% mutation level. Positive mutations were chosen such that the panel contained a representative mutation amplified by each of the four master mixes. Each operator tested the panel using 2 lots of grouped reagents–2 lots of Abbott *m*Sample Preparation System$_{DNA}$ kits, Abbott RealTi*me* IDH2 Amplification Reagent Kits, and Abbott RealTi*me* IDH2 Control Kits–for 4 runs per lot over 4 days. Each run consisted of 2 replicates for each panel member for a total of 32 replicates per panel member (16 replicates per lot). Testing was conducted using 2 *m*2000*rt* instruments. Each reagent lot was assigned to its own instrument.

All IDH2 mutation positive panel members reported "Mutation Detected" with a 100% percent agreement across all 32 replicates, and all IDH2 mutation negative panel members reported "Not Detected" across all 32 replicates. The percent agreement by operator was 100% (16/16) agreement for each panel member. The percent agreement by lot was 100% (16/16) agreement for each panel member. The Abbott RealTi*me* IDH2 assay demonstrated a percent agreement of 100% with expected results for each panel member by operator, by lot, and overall.

In within-laboratory repeatability, the mean and SD of dCN values and the within-run, between-run, between-lot and between-operator variability are shown in **Table 6**. The mean and SD of IC CN values and the within-run, between-run, between-lot and between-operator variability for Positive and Negative Controls are shown in **Table 7**. The mean and SD of mutant CN values and the within-run, between-run, between-lot and between-operator variability for Positive Controls are shown in **Table 8**.

Table 6. Analysis of dCN Values for Mutation Positive Panel Members

					Within-Run Variability		Between-Run Variability		Between-Lot Variability		Between-Operator Variability		Total	
Panel	Mutation Status	Sample Type	N	Mean	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
03	R140Q	Blood	32	5.06	0.129	2.5	0.088	1.7	0.061	1.2	0.210	4.2	0.264	5.2
04	R140W	Blood	32	5.70	0.091	1.6	0.138	2.4	0.302	5.3	0.302	5.3	0.345	6.1
05	R172K	Blood	32	3.01	0.125	4.1	0.017	0.6	0.226	7.5	0.000	0.0	0.259	8.6
06	R172W	Blood	32	4.97	0.079	1.6	0.158	3.2	0.208	4.2	0.208	4.2	0.273	5.5
09	R140Q	Bone Marrow	32	5.49	0.126	2.3	0.153	2.8	0.185	3.4	0.031	0.6	0.271	4.9
10	R140W	Bone Marrow	32	5.67	0.328	5.8	0.117	2.1	0.269	4.8	0.138	2.4	0.440	7.8
11	R172K	Bone Marrow	32	3.75	0.172	4.6	0.207	5.5	0.113	3.0	0.206	5.5	0.358	9.5
12	R172W	Bone Marrow	32	4.33	0.064	1.5	0.318	7.3	0.302	7.0	0.302	7.0	0.443	10.2

Table 7. Analysis of Oligo IC CN Values for Positive and Negative Controls

Panel	Value	N	Mean	Within-Run Variability SD	Between-Run Variability SD	Between-Lot Variability SD	Between-Operator Variability SD	Total SD
IDH2 Negative Control	OLIGO1 IC CN	32	22.79	0.264	0.000	0.059	0.000	0.270
	OLIGO2 IC CN	32	22.78	0.253	0.000	0.076	0.000	0.265
	OLIGO3 IC CN	32	22.76	0.291	0.000	0.034	0.008	0.293
	OLIGO4 IC CN	32	22.81	0.283	0.000	0.071	0.000	0.292
IDH2 Positive Control	OLIGO1 IC CN	32	22.83	0.303	0.000	0.141	0.040	0.337
	OLIGO2 IC CN	32	22.82	0.320	0.000	0.130	0.051	0.349
	OLIGO3 IC CN	32	22.81	0.305	0.000	0.138	0.043	0.338
	OLIGO4 IC CN	32	22.83	0.290	0.041	0.139	0.051	0.329

Table 8. Analysis of Mutant CN Values for Positive Control

Panel	Value	N	Mean	Within-Run Variability SD	Between-Run Variability SD	Between-Lot Variability SD	Between-Operator Variability SD	Total SD
IDH2 Positive Control	R140Q CN	32	26.03	0.347	0.000	0.290	0.096	0.462
	R140W CN	32	25.98	0.332	0.029	0.541	0.056	0.638
	R172K CN	32	23.74	0.313	0.000	0.000	0.000	0.313
	R172W CN	32	25.16	0.239	0.091	0.593	0.019	0.645

Within-Laboratory Lot-to-Lot Reproducibility

The within-laboratory lot-to-lot reproducibility was evaluated using 3 unique lots of Abbott *m*Sample Preparation System$_{DNA}$ kits and 3 unique lots of Abbott RealTi*me* IDH2 Amplification Reagent Kits. A 12-member panel contained IDH2 mutation negative blood and bone marrow specimens as well as blood and bone marrow specimens spiked with R140Q, R140W, R172K, or R172W positive cell lines targeting a 6% mutation level. Positive mutations were chosen such that the panel contained a representative mutation amplified by each of the four master mixes. For each panel member, 10 replicates were prepared using 3 unique lots of Abbott *m*Sample Preparation System$_{DNA}$ kits for a total of 30 extractions per panel member. Each extraction was tested using 3 unique lots of Abbott RealTi*me* IDH2 Amplification Reagent Kit for a total of 9 unique sample preparation/amplification kit combinations. Three *m*2000*rt* instruments were used, one for each lot of Abbott RealTi*me* IDH2 Amplification Reagent Kit. Thus, for each panel member, a total of 90 replicates were tested.

All mutation positive panel members reported "Mutation Detected" with a 100% percent agreement across all 90 replicates, and all mutation negative panel members reported "Not Detected" with a 100% percent agreement across all 90 replicates . The percent agreement by Abbott *m*Sample Preparation System$_{DNA}$ lot was 100% (30/30) agreement for each panel member. The percent agreement by Abbott RealTi*me* IDH2 Amplification Reagent Kit lot was 100% (30/30) agreement for each panel member. The Abbott RealTi*me* IDH2 assay demonstrated a percent agreement of 100% with expected results for each panel member by sample preparation kit lot, amplification reagent kit lot, and overall.

In within-laboratory lot-to-lot reproducibility, the mean and SD of dCN values and the between-replicate, between-amplification lot and between-sample prep lot variability are shown in **Table 9**. The mean and SD of IC CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive and Negative Controls is shown in **Table 10**. The mean and SD of mutant CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive and Negative Controls is shown in **Table 11**.

Table 9. Analysis of dCN Values

Panel	Mutation Status	Sample Type	N	Mean	Between-Replicate Variability SD	Between-Amplification Lot Variability SD	Between-Sample Prep Lot Variability SD	Total SD
03	R140Q	Blood	90	5.01	0.168	0.188	0.082	0.265
04	R140W	Blood	90	5.49	0.173	0.256	0.176	0.331
05	R172K	Blood	90	2.86	0.139	0.076	0.160	0.225
06	R172W	Blood	90	4.93	0.084	0.151	0.063	0.184
09	R140Q	Bone Marrow	90	4.48	0.212	0.200	0.209	0.358
10	R140W	Bone Marrow	90	5.90	0.134	0.201	0.029	0.242
11	R172K	Bone Marrow	90	3.81	0.195	0.068	0.091	0.226
12	R172W	Bone Marrow	90	4.38	0.205	0.162	0.064	0.269

Table 10. Analysis of IC CN Values for Positive and Negative Controls

Panel	Value	N	Mean	Between-Replicate Variability SD	Between-Amplification Lot Variability SD	Between-Sample Prep Lot Variability SD	Total SD
IDH2 Negative Control	OLIGO1 IC CN	54	23.13	0.387	0.460	0.000	0.601
	OLIGO2 IC CN	54	23.19	0.567	0.548	0.000	0.788
	OLIGO3 IC CN	54	23.16	0.569	0.544	0.000	0.787
	OLIGO4 IC CN	54	23.25	0.617	0.616	0.041	0.873
IDH2 Positive Control	OLIGO1 IC CN	54	23.14	0.394	0.485	0.095	0.632
	OLIGO2 IC CN	54	23.16	0.381	0.510	0.086	0.643
	OLIGO3 IC CN	54	23.14	0.426	0.556	0.121	0.711
	OLIGO4 IC CN	54	23.20	0.376	0.513	0.098	0.643

Table 11. Analysis of Mutant CN Values for Positive Control

Panel	Value	N	Mean	Between-Replicate Variability SD	Between-Amplification Lot Variability SD	Between-Sample Prep Lot Variability SD	Total SD
IDH2 Positive Control	R140Q CN	54	26.22	0.379	0.330	0.108	0.514
	R140W CN	54	26.03	0.402	0.484	0.149	0.646
	R172K CN	54	24.10	0.465	0.472	0.130	0.675
	R172W CN	54	25.31	0.371	0.438	0.101	0.583

Within-Laboratory Specimen Handling Reproducibility

The within-laboratory specimen handling reproducibility of Abbott RealTi*me* IDH2 was evaluated using two operators. Each operator tested a 4-member panel containing two mutation positive AML blood specimens and two mutation positive AML bone marrow aspirate specimens. Each operator tested the panel using the same lots of Abbott RealTi*me* IDH2 Amplification Reagent Kit and Abbott RealTi*me* IDH2 Control Kit and unique lots of Abbott *m*Sample Preparation System$_{DNA}$ Kits. Each operator performed 1 run per day over a total of 5 days. A run consisted of sample preparation and PCR. Two (2) replicates of each panel member were tested in each run, for a total of 20 replicates per panel member (10 replicates per operator). One (1) replicate of each control was tested in each run, for a total of 10 replicates per control (5 replicates per operator). Testing was conducted using 2 *m*2000*rt* instruments. Each Abbott *m*Sample Preparation System$_{DNA}$ Kit lot was assigned to its own instrument.

In the within-laboratory specimen handling reproducibility, all IDH2 mutation positive panel members were reported as "Mutation Detected" with 100% (10/10) agreement for each panel member by operator, and an overall agreement of 100% (20/20) for each panel member. The mean and SD of dCN values and the within-run, between-run and between-operator variability are shown in **Table 12**. The mean and SD of IC CN values and the between-run and between-operator variability for Positive and Negative Controls are shown in **Table 13**. The mean and SD of mutant CN values and the between-run and between-operator variability for Positive and Negative Controls are shown in **Table 14**.

Table 12. Analysis of dCN Values for Panel Members

Panel	Value	Mutation Status	Sample Type	N	Mean (dCN)	Within-Run Variability SD	Between-Run Variability SD	Between-Operator Variability SD	Total SD
1	dCN	R140W	Bone Marrow	20	8.15	0.209	0.000	0.000	0.209
2	dCN	R140Q	Bone Marrow	20	2.13	0.038	0.038	0.059	0.080
3	dCN	R172K	Blood	20	1.03	0.034	0.039	0.157	0.165
4	dCN	R140W	Blood	20	7.97	0.078	0.060	0.382	0.395

Table 13. Analysis of Oligo IC CN Values for Positive and Negative Controls

Control	Value	N	Mean (CN)	Between-Run Variability SD	Between-Operator Variability SD	Total SD
IDH2 Negative Control	OLIGO1 IC CN	10	22.49	0.187	0.325	0.375
	OLIGO2 IC CN	10	22.51	0.185	0.306	0.357
	OLIGO3 IC CN	10	22.47	0.223	0.344	0.409
	OLIGO4 IC CN	10	22.58	0.220	0.359	0.421
IDH2 Positive Control	OLIGO1 IC CN	10	23.01	0.114	0.313	0.333
	OLIGO2 IC CN	10	22.96	0.087	0.229	0.245
	OLIGO3 IC CN	10	22.97	0.103	0.243	0.264
	OLIGO4 IC CN	10	22.98	0.131	0.275	0.305

Table 14. Analysis of Mutant CN Values for Positive Control

Control	Value	N	Mean (CN)	Between-Run Variability SD	Between-Operator Variability SD	Total SD
IDH2 Positive Control	R140Q CN	10	25.65	0.114	0.363	0.380
	R140W CN	10	26.46	0.187	0.387	0.430
	R172K CN	10	23.45	0.112	0.400	0.415
	R172W CN	10	25.31	0.135	0.304	0.332

Reproducibility I

Reproducibility of the Abbott RealTi*me* IDH2 assay was evaluated at 3 external sites by testing IDH2 wild type (WT) mutation negative and IDH2 mutation positive blood and bone marrow specimens. The 6 member panel contained IDH2 wild type (WT) mutation negative and IDH2 R140Q and IDH2 R172K mutation positive blood and bone marrow specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. At each site, an operator performed 1 extraction run of the testing panel for 6 days. Each run contained 2 replicates of each panel member yielding a total of 12 replicates for each panel member or 36 replicates for each panel member for all 3 sites combined. Each run contained 1 replicate of each control yielding a total of 6 replicates for each control for 18 replicates for each control for all 3 sites combined. Each site used a unique lot of the Abbott *m*Sample Preparation System$_{DNA}$ kit for a total of 3 unique lots. The same lot of Abbott RealTi*me* IDH2 Amplification Reagent Kit and Abbott RealTi*me* IDH2 Control Kit was used across all sites. The sample handling reproducibility analysis demonstrated 100% (12/12) agreement for each panel member by site, and 100% (36/36) overall agreement for each panel member.

In Reproducibility I, the mean and SD of dCN values and the within-run, between-run and between-site variability are shown in **Table 15**. The mean and SD of IC CN and mutant CN and the between-run and between-site variability for Positive and Negative Controls are shown in **Table 16**.

Table 15. Reproducibility I Overall Precision

Panel	Mutation	Specimen Type	Value	N	Mean	Within-Run Variability SD	Between-Run Variability SD	Between-Site Variability SD	Total SD
1	WT	Blood	OLIGO1 IC CN	36	16.05	1.578	0.000	1.383	2.099
	WT	Blood	OLIGO2 IC CN	36	16.04	1.628	0.000	1.392	2.142
	WT	Blood	OLIGO3 IC CN	36	15.96	1.545	0.000	1.371	2.066
	WT	Blood	OLIGO4 IC CN	36	16.06	1.642	0.000	1.363	2.134
2	R140Q	Blood	dCN	36	4.13	0.148	0.106	0.187	0.261
3	R172K	Blood	dCN	36	2.17	0.111	0.094	0.219	0.263
4	WT	Bone Marrow	OLIGO1 IC CN	36	17.54	0.306	0.203	0.562	0.671
	WT	Bone Marrow	OLIGO2 IC CN	36	17.52	0.298	0.206	0.564	0.670
	WT	Bone Marrow	OLIGO3 IC CN	36	17.44	0.279	0.229	0.551	0.659
	WT	Bone Marrow	OLIGO4 IC CN	36	17.54	0.288	0.193	0.543	0.644
5	R140Q	Bone Marrow	dCN	36	4.52	0.163	0.091	0.212	0.282
6	R172K	Bone Marrow	dCN	36	2.69	0.203	0.093	0.144	0.266

Table 16. Reproducibility I Analysis of Oligo IC CN and Mutant CN Values for Positive and Negative Controls

Control	Value	N	Mean	Between-Run Variability SD	Between-Site Variability SD	Total SD
IDH2 Negative Control	OLIGO1 IC CN	18	22.99	0.177	0.087	0.198
	OLIGO2 IC CN	18	22.93	0.174	0.113	0.208
	OLIGO3 IC CN	18	22.87	0.159	0.117	0.197
	OLIGO4 IC CN	18	22.94	0.182	0.097	0.206
IDH2 Positive Control	R140Q CN	18	25.07	0.282	0.215	0.354
	R140W CN	18	25.73	0.235	0.000	0.235
	R172K CN	18	23.03	0.150	0.208	0.256
	R172W CN	18	24.97	0.162	0.060	0.173
	OLIGO1 IC CN	18	22.37	0.194	0.000	0.194
	OLIGO2 IC CN	18	22.29	0.163	0.000	0.163
	OLIGO3 IC CN	18	22.25	0.172	0.089	0.194
	OLIGO4 IC CN	18	22.38	0.182	0.109	0.212

Reproducibility II

Reproducibility of the Abbott RealTi*me* IDH2 assay was evaluated at 3 external sites by testing DNA eluate extracted from IDH2 wild type (WT) mutation negative and IDH2 mutation positive blood and bone marrow specimens. The panel members included 10 IDH2 SNVs and wild type specimens targeting 50 ng and 200 ng gDNA input levels. IDH2 mutation positive panel members were prepared to target a mutation percentage of approximately 2% or 15%. The R140Q and R172K panel members for both blood and bone marrow were prepared using clinical specimens. The remaining mutation positive panel members were prepared from eluates of IDH2 mutation positive cell lines spiked into blood or bone marrow. Panel members were tested using 3 unique lots of Abbott RealTi*me* IDH2 Amplification Reagent Kit. At each site, 2 operators performed 2 amplification/detection runs over a minimum of 5 days. Each run included 3 replicates of each panel member yielding a total of 180 replicates for each panel member for all 3 sites combined. There was a total of 241 replicates of each control for all 3 sites combined, which included 4 replicates of each control per run, plus 1 additional plate run by 1 site.

The eluate reproducibility analysis demonstrated 100% (60/60) agreement for each panel member at each site and 100% (60/60) agreement for each panel member tested with each Abbott RealTi*me* Amplification Reagent Kit lot. The overall agreement was 100% (180/180) for each panel member for all sites and lots combined.

In Reproducibility II, the mean and SD of dCN values and the within-run, between-run, between-lot, between-operator and between-site variability are shown in **Table 17**. The mean and SD of IC CN and mutant CN values and the between-run, between-lot, between-operator and between-site variability for Positive and Negative Controls are shown in **Table 18**.

Table 17. Reproducibility II Overall Precision

Panel	Mutation	Specimen Type	Value	N	Mean	Within-Run Variability SD	Between-Run Variability SD	Between-Lot Variability SD	Between-Operator Variability SD	Between-Site Variability SD	Total SD
1	R140Q	Bone Marrow	dCN	180	6.25	0.259	0.074	0.092	0.083	0.235	0.378
2	R172K	Bone Marrow	dCN	180	0.61	0.047	0.040	0.055	0.015	0.317	0.328
3	R140W	Bone Marrow	dCN	180	6.80	0.190	0.149	0.143	0.072	0.229	0.369
4	R140L	Bone Marrow	dCN	180	6.65	0.191	0.030	0.025	0.032	0.084	0.215
5	R140G	Bone Marrow	dCN	180	4.65	0.113	0.053	0.081	0.002	0.348	0.378
6	R172M	Bone Marrow	dCN	180	3.73	0.085	0.035	0.014	0.000	0.127	0.157
7	R172G	Bone Marrow	dCN	180	6.04	0.195	0.000	0.090	0.000	0.110	0.242
8	R172Sc	Bone Marrow	dCN	174[a]	0.85	0.062	0.020	0.050	0.026	0.284	0.297
9	R172St	Bone Marrow	dCN	180	4.03	0.143	0.000	0.046	0.019	0.303	0.339
10	R172W	Bone Marrow	dCN	180	3.35	0.083	0.025	0.042	0.027	0.122	0.158
11	R140Q	Blood	dCN	179[b]	3.46	0.112	0.064	0.028	0.071	0.276	0.314
12	R172K	Blood	dCN	180	3.70	0.107	0.045	0.054	0.000	0.342	0.365
13	R140W	Blood	dCN	180	6.89	0.276	0.137	0.000	0.153	0.407	0.533
14	R140L	Blood	dCN	180	6.84	0.118	0.055	0.102	0.023	0.125	0.208

Panel	Mutation	Specimen Type	Value	N	Mean	Within-Run Variability SD	Between-Run Variability SD	Between-Lot Variability SD	Between-Operator Variability SD	Between-Site Variability SD	Total SD
15	R140G	Blood	dCN	174[a]	4.73	0.141	0.040	0.089	0.000	0.326	0.368
16	R172M	Blood	dCN	180	6.31	0.169	0.030	0.018	0.000	0.127	0.214
17	R172G	Blood	dCN	174[a]	3.07	0.112	0.000	0.071	0.000	0.068	0.149
18	R172Sc	Blood	dCN	180	3.78	0.147	0.000	0.047	0.054	0.287	0.330
19	R172St	Blood	dCN	179[b]	1.26	0.053	0.034	0.054	0.015	0.296	0.308
20	R172W	Blood	dCN	180	6.09	0.140	0.051	0.020	0.000	0.166	0.224
21	WT	Bone Marrow	OLIGO1 IC CN	180	18.61	0.138	0.129	0.000	0.010	0.197	0.274
	WT	Bone Marrow	OLIGO2 IC CN	180	18.61	0.139	0.105	0.000	0.000	0.183	0.253
	WT	Bone Marrow	OLIGO3 IC CN	180	18.55	0.164	0.110	0.000	0.053	0.193	0.281
	WT	Bone Marrow	OLIGO4 IC CN	180	18.63	0.080	0.124	0.000	0.044	0.216	0.265
22	WT	Blood	OLIGO1 IC CN	180	19.21	0.661	0.000	0.166	0.401	0.000	0.791
	WT	Blood	OLIGO2 IC CN	180	19.19	0.701	0.146	0.154	0.252	0.180	0.796
	WT	Blood	OLIGO3 IC CN	180	19.17	0.652	0.156	0.138	0.287	0.142	0.755
	WT	Blood	OLIGO4 IC CN	180	19.22	0.551	0.306	0.189	0.266	0.232	0.747

[a] Missing replicates due to operator error.
[b] Replicates excluded due to instrument error.

Table 18. Reproducibility II Analysis of Oligo IC CN and Mutant CN Values for Positive and Negative Controls

Control	Value	N	Mean	Between-Run Variability SD	Between-Lot Variability SD	Between-Operator Variability SD	Between-Site Variability SD	Total SD
IDH2 Negative Control	OLIGO1 IC CN	241	22.93	0.309	0.000	0.269	0.000	0.409
	OLIGO2 IC CN	241	22.91	0.314	0.000	0.245	0.000	0.398
	OLIGO3 IC CN	241	22.86	0.292	0.035	0.273	0.000	0.402
	OLIGO4 IC CN	241	22.95	0.283	0.000	0.298	0.000	0.411
IDH2 Positive Control	R140Q CN	241	25.60	0.356	0.000	0.302	0.000	0.467
	R140W CN	241	26.08	0.423	0.174	0.274	0.000	0.533
	R172K CN	241	23.50	0.296	0.045	0.277	0.223	0.466
	R172W CN	241	25.28	0.285	0.051	0.269	0.000	0.395
	OLIGO1 IC CN	241	22.79	0.327	0.000	0.283	0.000	0.432
	OLIGO2 IC CN	241	22.78	0.312	0.013	0.251	0.000	0.400
	OLIGO3 IC CN	241	22.75	0.318	0.040	0.281	0.000	0.426
	OLIGO4 IC CN	241	22.84	0.305	0.000	0.277	0.000	0.412

COMPARISON TO NGS

A retrospective correlation analysis was performed using a data set of 173 clinical patients with valid IDH2 mutation testing results from both the Abbott RealTi*me* IDH2 assay and one of two laboratory validated NGS (Next Generation Sequencing) methods. The data set included IDH2 mutation positive patients and IDH2 mutation negative patients, with similar numbers of blood and bone marrow samples from the 173 patients (167 blood samples and 150 bone marrow samples). The analysis evaluated agreement for the 9 IDH2 mutations that the Abbott RealTi*me* IDH2 assay is designed to detect.

The correlation analysis of the 173 patient results, using the Abbott RealTi*me* IDH2 assay and NGS assay qualitative interpretation ("Mutation Detected" or "Not Detected") as end points, is shown in **Table 19**. The analysis demonstrated a 100.00% (93/93) Positive Percent Agreement (PPA) with a 95% exact CI of (96.11%, 100.00%). The analysis demonstrated a 98.75% (79/80) Negative Percent Agreement (NPA) with a 95% exact CI of (93.23%, 99.97%).

The correlation of interpretation analysis was performed by sample type. For the blood sample type, the analysis demonstrated a 100.00% (91/91) Positive Percent Agreement (PPA) with a 95% exact CI of (96.03%, 100.00%). The analysis demonstrated a 98.68% (75/76) Negative Percent Agreement (NPA) with a 95% exact CI of (92.89%, 99.97%). For the bone marrow sample type, the analysis demonstrated a 100.00% (78/78) Positive Percent Agreement (PPA) with a 95% exact CI of (95.38%, 100.00%). The analysis demonstrated a 100.00% (72/72) Negative Percent Agreement (NPA) with a 95% exact CI of (95.01%, 100.00%).

An analysis demonstrating mutation identity agreement for samples identified as IDH2 "Mutation Detected" by the Abbott RealTi*me* IDH2 assay is shown in **Table 20**. There were no samples with mutations R140G, R140L, R172G, R172M, R172S, or R172W identified in this data set.

Table 19. Correlation of Interpretation of Abbott RealTi*me* IDH2 Results with NGS Method Results

	NGS Method		
Abbott RealTi*me* IDH2	Mutation Detected	Mutation Not Detected	Total
Mutation Detected	93	1	94
Mutation Not Detected	0	79	79
Total	93	80	173

Table 20. Correlation of Mutation Identity of Abbott RealTi*me* IDH2 Results with NGS Method Results

	NGS Method				
Abbott RealTi*me* IDH2	Not Detected	R140Q	R140W	R172K	Total
R140Q	0	72	0	0	72
R140W	0	0	1	0	1
R172K	1	1	0	19	21
Total	1	73	1	19	94

CONCORDANCE BETWEEN BLOOD AND BONE MARROW SPECIMEN TYPES

The Abbott RealTi*me* IDH2 assay is intended to be used on either blood or bone marrow aspirate. An analysis was performed to show concordance of results between matched blood and bone marrow specimen types tested by Abbott RealTi*me* IDH2. Concordance between bone marrow specimens and blood specimens was determined at the subject level ("Mutation Detected" vs. "Not Detected"). Results are shown below in **Table 21**. There was one discordant call (R172K positive in blood was reported as mutation not detected in bone marrow). There was also one within-mutation detected discordant case (R140Q positive result in blood was reported as R172K positive in bone marrow (data not shown)). These results demonstrate equivalency between specimen types.

Table 21. Abbott RealTi*me* IDH2 Concordance of Results from Blood versus Bone Marrow

	Blood		
Bone Marrow	Mutation Detected	Not Detected	Total
Mutation Detected	142	1	143
Not Detected	0	70	70
Total	142	71	213

Average Positive Agreement = 99.65% (284/285); 95% CI = 98.87 – 100.00%
Average Negative Agreement = 99.29% (140/141); 95% CI = 97.58 – 100.00%
Overall Agreement = 99.53% (212/213); 95% CI = 97.41 – 99.99%

CLINICAL STUDIES

The safety and effectiveness of the Abbott RealTi*me* IDH2 assay were demonstrated through testing of specimens from patients enrolled in Study AG221-C-001 (ClinicalTrials.gov Identifier: NCT01915498). Study AG221-C-001 was an open-label, single-arm, international, multicenter, two-cohort clinical trial of IDHIFA (enasidenib) on 199 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 9 IDH2 mutations in codons R140 or R172, who were assigned to receive a 100 mg daily dose. Cohort 1 included 101 patients and Cohort 2 included 98 patients. IDH2 mutations were identified by a local diagnostic test and retrospectively confirmed by the Abbott RealTi*me* IDH2 assay, or prospectively identified by the Abbott RealTi*me* IDH2 assay. IDHIFA was given orally at starting dose of 100 mg daily until disease progression or unacceptable toxicity. Dose reductions were allowed to manage adverse events.

The baseline demographics and disease characteristics were similar in both study cohorts. The median age of study participants was 68 years and predominantly white (77%). Enrollment according to gender was equivalent. The predominant IDH2 positive mutation was R140Q (75%) followed by R172K (20%). Four (4) IDH2 mutations were not detected in patients enrolled into the trial (R140G, R172M, R172G, R172S).

Blood and bone marrow aspirate specimens from R/R AML patients being considered for treatment were tested. Patients with an IDH2 mutation positive result were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients with an IDH2 mutation negative result were ineligible for drug trial enrollment. Patients in Cohort 2 were selected on the basis of the Abbott RealTi*me* IDH2 assay. Patients in Cohort 1 used to support conclusions regarding IDHIFA efficacy were based on the IDH2 mutation positive specimen results as detected by the Abbott RealTi*me* IDH2 assay.

Efficacy for IDHIFA was established on the basis of the rate of complete response (CR)/complete response with partial hematologic recovery (CRh), the duration of CR/CRh, and the rate of conversion from transfusion dependence to transfusion independence. The efficacy results are shown in **Table 22** and were similar in both cohorts. The median follow-up was 6.6 months (range, 0.4 to 27.7 months). Similar CR/CRh rates were observed in patients with either R140 or R172 mutation.

Table 22. Efficacy Results in Patients with Relapsed or Refractory Acute Myeloid Leukemia (AML)

Endpoint	**IDHIFA (100 mg daily) N=199**
CR[a] n (%)	37 (19)
95% CI	(13, 25)
Median DOR[b] (months)	8.2
95% CI	(4.7, 19.4)
CRh[c] n (%)	9 (4)
95% CI	(2, 8)
Median DOR (months)	9.6
95% CI	(0.7, NA)
CR/CRh n (%)	46 (23)
95% CI	(18, 30)
Median DOR (months)	8.2
95% CI	(4.3, 19.4)

CI: confidence interval, NA: not available

[a] CR (complete remission) was defined as <5% of blasts in the bone marrow, no evidence of disease, and full recovery of blood counts (platelets >100,000/microliter and absolute neutrophil counts [ANC] >1,000/microliter).

[b] DOR (duration of response) was defined as time since first response of CR or CRh to relapse or death, whichever is earlier.

[c] CRh (complete remission with partial hematological recovery) was defined as <5% of blasts in the bone marrow, no evidence of disease, and partial recovery of blood counts (platelets >50,000/microliter and ANC >500/microliter).

For patients who achieved a CR/CRh, the median time to first response was 1.9 months (range, 0.5 to 7.5 months) and the median time to best response of CR/CRh was 3.7 months (range, 0.6 to 11.2 months). Of the 46 patients who achieved a best response of CR/CRh, 39 (85%) did so within 6 months of initiating IDHIFA.

Among the 157 patients who were dependent on red blood cell (RBC) and/or platelet transfusions at baseline, 53 (34%) became independent of RBC and platelet transfusions during any 56-day post baseline period. Of the 42 patients who were independent of both RBC and platelet transfusions at baseline, 32 (76%) remained transfusion independent during any 56-day post baseline period.

Refer to Drugs@FDA for the most recent IDHIFA product labeling.

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TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 or visit the Abbott Molecular Web site at http://www.abbottmolecular.com.

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Abbott RealTi*me* IDH2 Amplification Reagent Kit

(List No. 08N82-090)

Abbott RealTi*me* IDH2 Control Kit (List No. 08N82-080)

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Appendix 1. List of Abbott RealTi*me* IDH2 Specific Error Codes for Positive Control and Negative Control

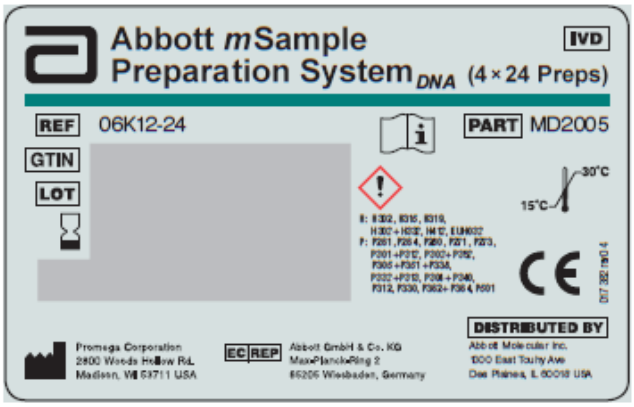
Code	Message Text	Probable Causes	Corrective Actions
4926	IC cycle number is less than the minimum	• Sample preparation reagents, master mix, or sample contaminated with targets • Sample preparation procedure errors • Wrong sample loaded onto reaction plate	1. Decontaminate work areas, pipettes, and equipment. 2. Refer to this package insert for further instructions on precautions to avoid contamination. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested.
4927	IC cycle number is greater than the maximum	• Insufficient DNA extracted • Insufficient reagent(s) pipetted • Sample preparation procedure errors • Wrong sample loaded onto reaction plate • No or insufficient sample loaded • Sample preparation reagent, master mix, or sample contaminated with DNase	1. Ensure that the correct amounts of Positive Control and Negative Control are used. 2. Ensure that calibrated pipettes and the correct amount of assay reagents are used. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested. 5. Ensure that the correct amount of eluate is used. 6. Follow package insert for good laboratory practices to avoid DNase contamination.
4928	Negative Control is reactive for a mutation	• Sample preparation reagent, master mix, or sample contaminated with targets • Positive Control was extracted • Sample preparation procedure errors • Wrong sample loaded onto reaction plate	1. Decontaminate work areas, pipettes, and equipment. 2. Refer to this package insert for further instructions on precautions for avoiding contamination. 3. Ensure that a new vial of Negative Control is used. 4. Ensure that the correct sample preparation procedure is followed. 5. Ensure that the sample is correctly labeled and tested.
4929	Mutation cycle number is less than the minimum	• Sample preparation reagents, master mix, or sample contaminated with targets • Sample preparation procedure errors • Wrong sample loaded	1. Decontaminate work areas, pipettes, and equipment. 2. Refer to this package insert for further instructions on precautions for avoiding contamination. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested.
4930	Mutation cycle number is greater than the maximum	• Insufficient DNA extracted • Negative Control was extracted • Insufficient reagent(s) pipetted • Sample preparation procedure errors • Wrong sample loaded onto reaction plate • No or insufficient sample loaded • Sample preparation reagent, master mix, or sample contaminated with DNase	1. Ensure that the correct amount of Positive Control is used. 2. Ensure that calibrated pipettes and the correct amount of assay reagents are used. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested. 5. Ensure that the correct amount of eluate is used. 6. Follow package insert for good laboratory practices to avoid DNase contamination.

Appendix 2. List of Abbott RealTi*me* IDH2 Specific Error Codes for Samples

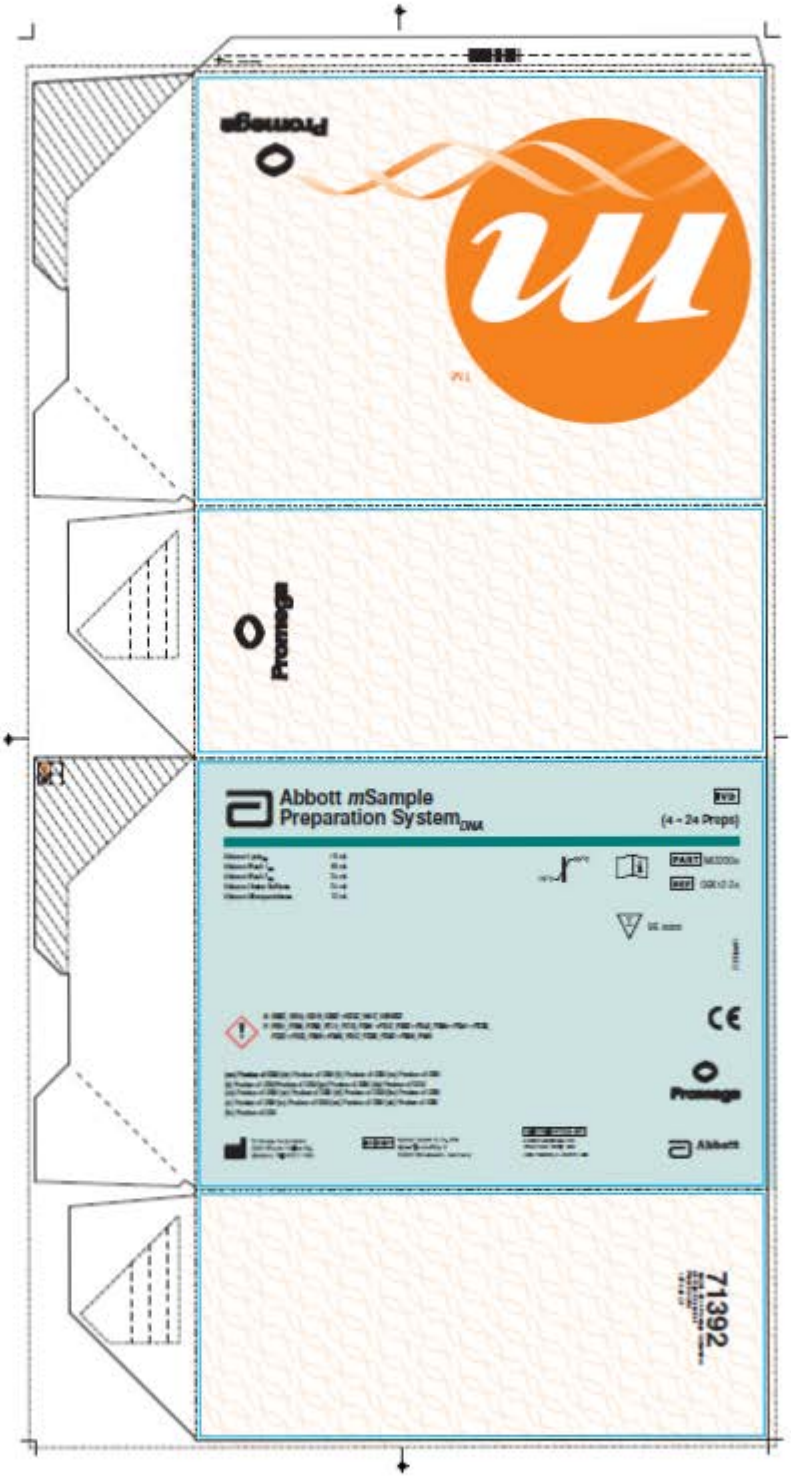
Code	Message Text	Probable Causes	Corrective Actions
4931	IC cycle number is non-reactive or greater than the maximum	• Poor sample quality • Insufficient DNA extracted • Insufficient reagent(s) pipetted • Sample preparation procedure errors • No or insufficient sample loaded • Sample preparation reagent, master mix, or sample contaminated with DNase	1. Ensure that the specimen meets requirements. 2. Ensure that calibrated pipettes and the correct amount of assay reagents are used. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the correct amount of eluate is used. 5. Follow package insert for good laboratory practices to avoid DNase contamination.
4932	IC cycle number is less than the minimum	• Sample preparation reagents, master mix, or sample contaminated with targets • Sample preparation procedure errors • Wrong sample loaded onto reaction plate	1. Decontaminate work areas, pipettes, and equipment. 2. Refer to this package insert for further instructions on precautions for avoiding contamination. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested.
4933	Cycle number difference is less than the minimum	• Poor sample quality • Sample preparation reagents, master mix, or sample contaminated with targets • Sample preparation procedure errors • Wrong sample loaded onto reaction plate	1. Decontaminate work areas, pipettes, and equipment. 2. Refer to this package insert for further instructions on precautions for avoiding contamination. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested.
4934	See Constituent Result for Associated Error	• Refer to Constituent Result error code[a]	Refer to Constituent Result error code[a]
4935	See Constituent Result for Associated Error	• Refer to Constituent Result error code[a]	Refer to Constituent Result error code[a]

[a] Constituent Result error codes are found associated with individual IC (OLIGO1, OLIGO2, OLIGO3, or OLIGO4) or mutation (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) results.

1.16 Abbott *m*Sample Preparation System $_{DNA}$ Label



1.17 Abbott *m*Sample Preparation System $_{DNA}$ Box



Abbott *m*Sample Preparation System$_{DNA}$ (4 × 24 Preps)

en

REF 06K12-24

PART MD2005

9PIMD2005rev03

In Vitro Test; For In Vitro Diagnostic Use

Key to Symbols used	
GTIN	Global Trade Item Number
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
PART	Part Number
DISTRIBUTED BY	Distributed By
	Do Not Reuse
	Contains sufficient for <*n*> tests
	Use By
	Temperature Limit
	Consult instructions for use
	Refer to **WARNINGS AND PRECAUTIONS**
EC REP	Authorized Representative in the European Community
	Manufacturer

CUSTOMER SERVICE: 1-800-553-7042

CUSTOMER SERVICE INTERNATIONAL: CALL YOUR ABBOTT REPRESENTATIVE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

PROPRIETARY NAME

Abbott *m*Sample Preparation System$_{DNA}$ (4 × 24 Preps)

INTENDED USE

The Abbott *m*Sample Preparation System$_{DNA}$ is used for the laboratory isolation of nucleic acids from biological samples that is functional in standard Polymerase Chain Reaction (PCR) reactions.

REAGENTS AND INSTRUMENTS

Materials Provided

1. **Abbott *m*Lysis$_{DNA}$:** (MD201A) (4 each, 70 mL) 100mM Tris solution containing Guanidinium thiocyanate and detergent.
2. **Abbott *m*Wash 1$_{DNA}$:** (MD202A) (4 each, 46 mL) 100mM Tris solution containing Guanidinium thiocyanate and detergent.
3. **Abbott *m*Wash 2$_{DNA}$:** (MD203A) (4 each, 25 mL) Nuclease free water.
4. **Abbott *m*Elution Buffer$_{DNA}$:** (MD204A) (4 each, 25 mL) Nuclease free water.
5. **Abbott *m*Microparticles$_{DNA}$:** (MD205A) (4 each, 13 mL) 8% microparticles in Guanidinium hydrochloride.

Materials Required But Not Provided

Refer to individual Abbott RealTi*me* assay package insert for all ancillary reagents, components, and equipment.

WARNINGS AND PRECAUTIONS

IVD

*m*Lysis$_{DNA}$ and *m*Wash 1$_{DNA}$ contain Guanidinium thiocyanate.

Abbott *m*Lysis$_{DNA}$ and Abbott *m*Wash 1$_{DNA}$

Warning
Hazard-determining components of labeling:
Guanidinium thiocyanate

H302+H332	Harmful if swallowed or if inhaled.
H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
P261	Avoid breathing mist/vapours/spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/ eye protection.
P271	Use only outdoors or in a well-ventilated area.
P273	Avoid release to the environment.
P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
P330	Rinse mouth.
P501	Dispose of contents/container in accordance with local regulations.

*m*Microparticles$_{DNA}$ contain Guanidine hydrochloride.

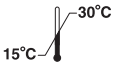
Abbott *m*Microparticles$_{DNA}$

Warning
Hazard-determining components of labeling:
Guanidine hydrochloride

H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
P280	Wear protective gloves/protective clothing/ eye protection.
P264	Wash hands thoroughly after handling.
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P302+P352	IF ON SKIN: Wash with plenty of water.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P330	Rinse mouth.
P362+P364	Take off contaminated clothing and wash it before reuse.
P501	Dispose of contents/container in accordance with local regulations.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

STORAGE INSTRUCTIONS



Store at 15°C to 30°C

ASSAY PROCEDURE

Refer to individual Abbott RealTi*me* assay package insert for detailed instruction on the sample preparation procedure.

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