PRODUCT INSTRUCTION MANUAL

QClamp® JAK2 Mutation Detection Test

Codon 617

CATALOG NUMBER

DC-10-1037R (10 Samples)
DC-10-0166R (30 Samples)
DC-10-0165R (60 Samples)

MANUFACTURER

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INTENDED USE

For Research Use Only

*Where appropriate, please disregard assay mixes that are not part of your kit

Document Number: MAN.0010 Instruction Version: Rev.2, Date of Revision: May 2022

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CONTENTS

PART 1. INTENDED USE 3

PART 2. JAK2 MUTATIONS AND CANCER* 3

PART 3. QCLAMP® WORKFLOW 3

PART 4. QCLAMP® TECHNOLOGY FOR MUTATION DETECTION 4

PART 5. REAGENTS AND INSTRUMENTS 4

PART 6. INSTRUCTIONS FOR USE 8

PART 7. ASSESSMENT OF QPCR RESULTS 10

PART 8. ASSAY PERFORMANCE CHARACTERISTICS 12

PART 9. SYMBOLS USED IN PACKAGING 14

REFERENCES 14

PART 1. INTENDED USE

The QClamp® JAK2 Mutation Detection Test is a real-time qPCR assay for the detection of somatic mutations in and near JAK2 codon 617 in Exon 14 in the JAK2 tyrosine kinase gene, using purified DNA extracted from plasma and tissue. The QClamp® JAK2 Mutation Detection Test kits are designed to detect any mutation at or near the stated codon site without specifying the exact nucleotide change. Table 1 shows the most common mutations found in the JAK2 gene that can be detected by the kit. The kit is to be used within a laboratory environment for research only, not for clinical diagnostic purposes.

Table 1. List of Mutations and Cosmic Identities Commonly Found in JAK2

Exon	Amino Acid Change	Nucleotide Change	Cosmic No.
14	V617>F	1849G>T	12600

PART 2. JAK2 MUTATIONS AND CANCER*

Janus kinase 2 (JAK2) is an intracellular tyrosine kinase that associates with the cytoplasmic domains of multiple cytokine receptors. Ligards binding to the receptor results in conformational changes that activate JAK2, resulting in phosphorylation of target proteins, including STATs and JAK2 itself. More than 50% of myeloproliferative neoplasms (MPNs) harbor the activating JAK2 V617F mutation. In addition, a subset of B cell acute lymphoblastic leukemia (B-ALL) with rearrangements of cytokine receptor–like factor 2 (CRLF2) have activating JAK2 mutations that primarily involve R683.

A high proportion (>50%) of patients with myeloproliferative disorders (MPD: polycythemia vera, essential thrombocythemia, idiopathic myelofibrosis) carry a dominant gain-of-function V617F mutation in the JH2 kinase-like domain of JAK2. This mutation leads to deregulation of the kinase activity, and thus to constitutive tyrosine phosphorylation activity. The V617F mutation seems to occur exclusively in hematopietic malignancies of the myeloid lineage.

Note: *Adapted from https://www.mycancergenome.org/

PART 3. QCLAMP® WORKFLOW

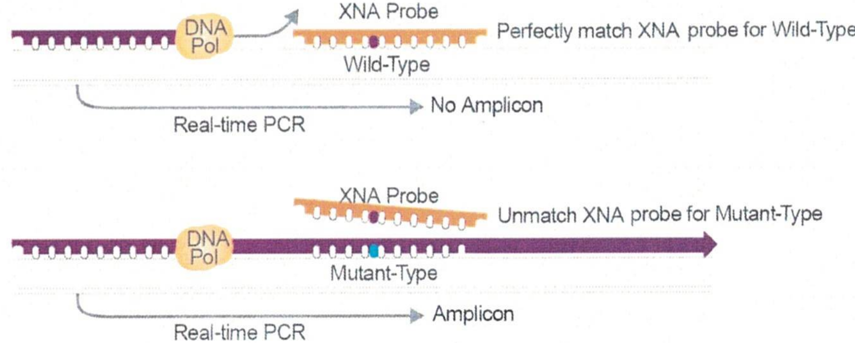
The assay workflow consists of four major steps:

1. **DNA Isolation:** Extract DNA from plasma and tissues using commercial DNA extraction kit
2. **Set up qPCR:** Mix the assay reagents, load into PCR plate, controls and extracted DNA sample ~ 30-60 minutes
3. **Enter amplification parameters** on qPCR machine, load PCR plate and start the run ~ 2.5 hours
4. **Data analysis:** Determine the presence or absence of mutations according to the Cq value cutoffs ~ 15 minutes

PART 4. QCLAMP® TECHNOLOGY FOR MUTATION DETECTION

The QClamp® JAK2 Mutation Detection Test is based on xenonucleic acid (XNA) mediated PCR clamping technology. XNA is a synthetic DNA analog in which the phosphodiester backbone has been replaced by a repeat formed by units of DiaCarta's proprietary novel uncharged backbone chemistry. XNAs hybridize tightly to complementary DNA target sequences only if the sequence is a complete match. Binding of XNA to its target sequence blocks strand elongation by DNA polymerase. When there is a mutation in the target site, and therefore a mismatch, the XNA:DNA duplex is unstable, allowing strand elongation by DNA polymerase. Addition of an XNA, whose sequence with a complete match to wild-type DNA, to a PCR reaction, blocks amplification of wild-type DNA allowing selective amplification of mutant DNA. XNA oligomers are not recognized by DNA polymerases and cannot be utilized as primers in subsequent qPCR reactions.

Figure 1. Principle of the QClamp® JAK2 Mutation Detection Test



PART 5. REAGENTS AND INSTRUMENTS

5.1. Package Contents

Table 2a. Package Contents – Pack Size: 10 samples (DC-10-1037R, DC-10-1037)

Name of Component	Part #	Description	Pack Size: 10 samples	Label Volume for each vial	Fill Volume for each vial	Cap Color	Storage Temp
Primer/Probe Mix	1007532	JAK2 c617 Primer and	1 vial	22 µL	28 µL	Amber	-25°C to -15°C
XNA	1007542	JAK2 c617 XNA	1 vial	22 µL	28 µL	Clear	-25°C to -15°C
2X qPCR Master Mix	1007572	PCR Reaction Premix	1 vial	110 µL	125 µL	Blue	-25°C to -15°C
Positive Control	1007552	JAK2 c617 Mutant Template	1 vial	6 µL	10 µL	Red	-25°C to -15°C
Negative Control	1007562	Wild type gDNA	1 vial	6 µL	10 µL	Green	-25°C to -15°C
Non-Template Control	1000592	Nuclease-Free Water	1 vial	72 µL	80 µL	Orange	-25°C to -15°C

Table 2b. Package Contents – Pack Size: 30 samples (DC-10-0166R, DC-10-0166)

Name of Component	Part #	Description	Pack Size: 30 samples	Label Volume for each vial	Fill Volume for each vial	Cap Color	Storage Temp
Primer/Probe Mix	1007533	JAK2 c617 Primer and	1 vial	54 µL	60 µL	Amber	-25°C to -15°C
XNA	1007543	JAK2 c617 XNA	1 vial	54 µL	60 µL	Clear	-25°C to -15°C
2X qPCR Master Mix	1007573	PCR Reaction Premix	1 vial	270 µL	300 µL	Blue	-25°C to -15°C
Positive Control	1007553	JAK2 c617 Mutant	1 vial	12 µL	16 µL	Red	-25°C to -15°C
Negative Control	1007563	Wild-type gDNA	1 vial	12 µL	16 µL	Green	-25°C to -15°C
Non-Template Control	1000593	Nuclease-Free Water	1 vial	189 µL	210 µL	Orange	-25°C to -15°C

Table 2c. Package Contents – Pack Size: 60 samples (DC-10-0165R, DC-10-0165)

Name of Component	Part #	Description	Pack Size: 60 samples	Label Volume for each vial	Fill Volume for each vial	Cap Color	Storage Temp
Primer/Probe Mix	1007534	JAK2 c617 Primer and Probe	1 vial	84 µL	95 µL	Amber	-25°C to -15°C
XNA	1007544	JAK2 c617 XNA	1 vial	84 µL	95 µL	Clear	-25°C to -15°C
2X qPCR Master Mix	1007574	PCR Reaction Premix	1 vial	420 µL	470 µL	Blue	-25°C to -15°C
Positive Control	1007554	JAK2 c617 Mutant Template	1 vial	24 µL	30 µL	Red	-25°C to -15°C
Negative Control	1007564	Wild-type gDNA	1 vial	24 µL	30 µL	Green	-25°C to -15°C
Non-Template Control	1000594	Nuclease-Free Water	1 vial	324 µL	360 µL	Orange	-25°C to -15°C

5.2. Materials Required but Not Provided in the Kit

A. Reagents for DNA Isolation

- QIAamp DSP DNA FFPE Tissue Kit (QIAGEN, Cat. No. 60404) or equivalent
- QIAamp Circulating Nucleic Acid Kit (QIAGEN, Cat. No. 55114) or equivalent
- QIAamp MiniElute ccfDNA Kit (QIAGEN, Cat. No. 55204) or equivalent
- DNeasy Blood & Tissue Kit (QIAGEN, Cat. No. 69504 or 69506) for tissue and blood specimens

B. Consumables

- DNase-free PCR tubes or plates
- Nuclease-free low-binding micro centrifuge tubes
- Nuclease-free pipet tips with aerosol barriers

C. Equipment

- Permanent marker
- qPCR instrument capable of detecting FAM and HEX fluorescent probes
- Dedicated pipettes* (adjustable) for sample preparation
- Dedicated pipettes* (adjustable) for PCR Master Mix preparation
- Dedicated pipettes* (adjustable) for dispensing of template DNA
- Micro centrifuge
- Bench top centrifuge* with rotor for 1.5 ml tubes
- Vortexer
- PCR rack
- Reagent reservoir

Note: Prior to use ensure that instruments highlighted with * have been maintained and calibrated according to the manufacturer's recommendations.

5.3. Instruments

The assays have been developed and validated on the instruments shown in the table below.

Table 3. List of Instruments Validated with This Kit

Company	Model
Roche	LightCycler® 480
Bio-Rad	CFX384
ABI	QuantStudio 5
ABI	7500

5.4. Handling and Storage

This kit is shipped on dry ice. If any component of the kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packaging note or the reagents, please contact DiaCarta or the local distributors as soon as possible.

The kit should be stored at recommended condition immediately upon receipt, in a constant-temperature refrigerator or freezer and protected from light. It is recommended to store Box 1 from -15°C to -25°C in a pre-amplification area. The controls (Box 2) should be stored at -15°C to -25°C in a postamplification (DNA template-handling) area. When stored under the specified storage conditions, the kit is stable until the stated expiration date. The kit can undergo up to 6 freeze-thaw cycles without affecting performance.

All reagents must be thawed at ambient temperature for a minimum of 30 minutes before use. Do not exceed 2 hours at ambient temperature. The Primer and Probe Mixes contain fluorophore labeled probes and should be protected from light. Attention should be paid to expiration dates and storage conditions printed in the box and labels of all components. Do not use expired or incorrectly stored components.

5.5. General Considerations

Effective use of qPCR tests requires good laboratory practices, including maintenance of equipment that is dedicated to molecular biology. Use nuclease-free lab ware (pipettes, pipette tips, reaction vials) and wear gloves when performing the assay. Use aerosol-resistant pipette tips for all pipetting steps to avoid cross contamination of the samples and reagents.

Prepare the assay mixes in designated pre-amplification areas using only equipment dedicated to this application. Add template DNA in a separate area (preferably a separate room). Use extreme caution to prevent DNase contamination that could result in degradation of the template DNA, or PCR carryover contamination, which could result in a false positive signal.

Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data.

5.6. Warnings and Precautions

- Use extreme caution to prevent contamination of PCR reactions with the positive and negative DNA controls provided.
- Minimize exposure of the 2X PCR Master Mix to room temperature for optimal amplification.
- Avoid overexposing the Primer Probe Mixes to light for optimal fluorescent signal.
- Use of non-recommended reagent volumes may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended volumes and concentrations of the target DNA sample may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended consumables with instruments may adversely affect test results.
- Do not re-use any remaining reagents after PCR amplification is completed.
- Additional validation testing by user may be necessary when using non-recommended instruments.
- Perform all experiments under proper sterile conditions using aseptic techniques.
- Perform all procedures using universal precautions.
- Wear personal protective equipment, including disposable gloves, throughout the assay procedure.
- Do not eat, drink, smoke, or apply cosmetics in areas where reagents or specimens are handled.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Discard all materials in a safe and acceptable manner, in compliance with all legal requirements.
- Dissolve reagents completely, then mix thoroughly by vortexing, except for Primer Probe Mixes and Master Mixes.
- If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately.
- Do not use components beyond the expiration date printed on the kit boxes.
- Do not mix reagents from different lots.
- Return all components to the appropriate storage condition after preparing the working reagents.
- Do not interchange vial or bottle caps, as cross-contamination may occur.
- Keep all the materials on ice when in use.
- Do not leave components out at room temperature for more than 2 hours.
- Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data.

PART 6. INSTRUCTIONS FOR USE

6.1. DNA Isolation

Human genomic DNA must be extracted from tissue and plasma prior to use. Several methods exist for DNA isolation. For consistency, we recommend using a commercial kit, such as Qiagen DNA extraction kit (DNeasy Blood and Tissue Kit, Cat. No. 69504 or 69506, for tissue and blood specimens; for cfDNA extraction from plasma use Qiagen cfDNA extraction kits, such as Cat. No. 55114 or beads-based cfDNA extraction kit (Cat. No. 55204). Follow the DNA isolation procedure according to manufacturer's protocol.

This QClamp® assay requires a total of 5-10 ng of DNA per sample. After DNA isolation, measure the concentration using fluorometric analysis (i.e. Qubit, Cat. No. Q33216) and dilute to 2.5-5 ng/µl. If using spectrophotometric analysis, make sure the A260/A230 value is greater than 2.0 and A260/A280 value between 1.8 and 2.0.

6.2. Preparation of Reagents

Thaw all Primer and Probe Mixes, XNAs, Positive Control, Negative Control, Non-template Control (NTC, Nuclease-Free Water) and 2X PCR Master Mix provided. Thaw all reaction mixes at room temperature for a minimum of 30 minutes. Vortex all components except the PCR Master Mix and Primer/ Probe Mix for 5 seconds and perform a quick spin. The PCR Master Mix and Primer/probe mix should be mixed gently by inverting the tube a few times. Prior to use, ensure that the PCR Master Mix is re-suspended by pipetting up and down multiple times. Do not leave kit components at room temperature for more than 2 hours. The PCR reactions are set up in a total volume of 10 µl/reaction.

- A 10-sample test kit contains enough material for 3 runs (minimum 3 samples per run).
- A 30-sample test kit contains enough material for 6 runs (minimum 5 samples per run).
- A 60-sample test kit contains enough material for 6 runs (minimum 10 samples per run).

Table 4. QClamp® Assay Components and Reaction Volume

Components	Volume/Reaction
2X PCR Master Mix	5 µl
Primer and Probe Mix	1 µl
XNA	1 µl
DNA Sample or Controls	2 µl
Nuclease Free Water	1 µl
Total Volume	**10 µl**

Notes:

- For accuracy, 2X PCR Master Mix, Primers and XNA should be pre-mixed into assay mixes as described in Table 5 below
- If needed, the sample volume can be increased to 3 µl by eliminating 1 µl water

6.3. Preparation of Assay Mixes

This test contains 1 assay as listed below.

Table 5. Preparation of Assay Mixes

	Volume of 2X PCR Master Mix	Volume of Primer Mix	Volume of XNA	Volume of Water
C617 Mix	5 µl x (n+1)	1 µl x (n+1)	1 µl x (n+1)	1 µl x (n+1)

Notes:

- n = number of reactions (DNA samples plus 3 controls). Prepare enough for 1 extra sample (n+1) to allow for sufficient overage for the PCR set.
- For accuracy, do not pipette less than 8 µl.

Assay mixes should be prepared just prior to use. Label a micro centrifuge tube (not provided) for the reaction mix available, as shown in Table 5. Please disregard any assay mixes listed that are not part of your kit.

A reaction mix containing all reagents except for the DNA samples or controls should be prepared for the total number of samples and controls to be tested in one run. The JAK2 Mixed Positive Control (POS), JAK2 Negative Control (NEG), and a Non-Template Control (NTC) should be included in each run. Negative Controls use wild-type DNA as the template. Wild-type DNA should have no mutations; therefore, the XNA probes will bind strongly, blocking the polymerase from making amplicons. However, the Internal Control Assay (HEX) with the Negative Control should make amplicons efficiently, providing another way to monitor performance of the primers, polymerase, and samples.

A set of Positive Controls must also be run with each reaction mix, every time the assay is run. Positive Control contains mutation; therefore, XNA probes will not bind, allowing amplification of the mutant template. Positive Controls must show the appropriate values for the reaction to be valid. A set of Non-Template Control (tube NTC) is run with each reaction mix every time when the assay is run. Nuclease-Free Water is used in the place of template. The NTC serves as a Negative Control and assesses potential contamination during assay set-up.

6.4. Suggested Run Layout (96-Well Plate, 384-Well Plate, Tube Strips, or Tubes)

Add 8 µl of the appropriate assay mix to the plate or tubes to ensure that each reaction has a total of 10 µl.

Table 6. Suggested Plate Layout

	1	2	3	4	5	6
A	NTC c617 Mix	POS c617 Mix	NEG c617 Mix	S1 c617 Mix	S2 c617 Mix	S3 c617 Mix

Notes:

- POS: Positive Control (Mixed Mutant DNA), NTC: Non-Template Control (Nuclease-Free Water), NEG: Negative Control (Wild-Type DNA), S1-3: Samples 1-3.
- When all reagents have been added to the plate, tightly seal the plate to prevent evaporation. Spin at 2000rpm for 1 minute to collect all the reagents. Place in the qPCR instrument immediately. Table 6 is a suggested plate set-up for a single experiment analyzing 3 unknown samples.

6.5. Instrument Set-Up

These parameters apply to Bio-Rad CFX384, Roche LightCycler® 480, ABI QuantStudio 5 Instruments. For Bio-Rad CFX384, the entire run is on default and no manual adjustment should be performed. Choose all channels when prompted.

For Applied Biosystems Platforms select NONE for the Passive Reference Dye. For Roche LightCycler® 480 the ramp rates should be adjusted according to the below table.

Table 7. Cycling Parameters

Step	Temperature (°C)	Time (Seconds)	Ramp Rates for Roche LightCycler® 480	Cycles	Data Collection
Preincubation	95	300	4	1	OFF
Denaturation	95	20	1.55	X50	OFF
XNA Annealing	70	40	1.75		OFF
Primer Annealing	66	30	1.2		OFF
Extension	72	30	1.4		FAM and HEX

PART 7. ASSESSMENT OF QPCR RESULTS

The qPCR instrument generates a cycle threshold (Cq) value for each sample. Cq is the cycle number at which a signal is detected above the set threshold for fluorescence. The lower the cycle number at which signal rises above background, the stronger the PCR reaction it represents (**please see MIQE Guidelines under References for more information).

- **Negative Control** uses wild-type DNA as the template. Wild-type DNA does not contain the targeted mutation; therefore the XNA probes will bind strongly, inhibiting the target amplification.
- In contrast, the **Internal Control** Assay assessed with HEX channel should make amplicons efficiently for the Negative Control, providing another way to monitor performance of the primers, polymerase, and sample DNA quality/quantity.
- The **Positive Control** contains mutations; therefore, XNA probes will not bind, allowing amplification of the mutanttemplate. Positive Controls must show the appropriate values in both HEX and FAM channels for the run to be valid.
- A set of **Non-Template Control (NTC)** is run with each assay mix every time the assay is run. Nuclease-Free Water is used in the place of template. The NTC serves as a Negative Control and assesses potential contamination during assay set-up.

7.1. Data Analysis

For Bio-Rad CFX384, select FAM and HEX channels, set the RFU threshold at 100 for both channels and export the data table.

For the Roche LightCycler® 480, open the Roche LightCycler® 480 SW 1.5.1.61, choose Dual Color Hydrolysis Probe and select Abs Quant/Fit Points for All Samples algorithm and set the threshold at 0.7 for both channels to analyze the run file data.

For ABI QuantStudio 5, under Properties select TaqMan and under Plate, select FAM for Target 1 and VIC for Target 2. For the Passive reference select NONE. Set the delta Rn for both targets at 10,000 and proceed to the data analysis.

The threshold can be adjusted as needed for each run based on the levels of noise.

7.1.1. Non-Template Controls

Verify that no amplification is observed in the Non-Template Controls (NTC) for each of the reaction mixes. Cq should be Undetermined. For each control or sample, calculate the difference in Cq value between the Mutation Assay and the Internal Control Assay as follows:

Cq difference (ΔCq) = Mutation Assay (FAM) Cq - Internal Control Assay (HEX) Cq.

7.1.2. Analysis of Negative and Positive Controls

For the assay to be valid, the Negative Control and Positive Control must meet the criteria in Table 8a-d.

Table 8a. Acceptable Values for Positive Controls and Negative Controls (Bio-Rad CFX384)

	Positive Control Acceptable Values	Negative Control Acceptable Values
c617	ΔCq ≤ 9.2	ΔCq > 13.0

Table 8b. Acceptable Values for Positive Controls and Clamping Controls (Roche LightCycler® 480)

	Positive Control Acceptable Values	Negative Control Acceptable Values
c617	ΔCq ≤ 7.8	ΔCq > 9.8

Table 8c. Acceptable Values for Positive Controls and Clamping Controls (ABI QuantStudio 5)

	Positive Control Acceptable Values	Negative Control Acceptable Values
c617	ΔCq ≤ 9.7	ΔCq > 10.4

Table 8d. Acceptable Values for Positive Controls and Clamping Controls (ABI 7500)

	Positive Control Acceptable Values	Negative Control Acceptable Values
c617	ΔCq ≤ 9.7	ΔCq > 12.5

7.1.3. Judging Validity of Sample Data Based on Internal Control Test Results (HEX)

The Cq value of the Internal Control Mix can serve as an indication of the purity and concentration of DNA. Thus, the validity of the test can be decided by the Cq value of the Internal Control Mix. Cq values of any sample with Internal Control Mix should be in the range of 24-32. If the Cq values fall outside this range, the test results should be considered invalid. The experiment should be repeated following the recommendations in Table 9.

Table 9. Acceptable Cq Ranges for Samples with Internal Control Mix

Validity	Cq Value of Int Control Mix	Descriptions and Recommendations
Optimal	24 < Cq < 32	The amplification and amount of DNA sample were optimal.
Invalid	Cq ≤ 24	Possibility of a false positive is high. Repeat the PCR reaction with less DNA.
Invalid	Cq ≥ 32	Not enough DNA or DNA not pure. The amplification is not optimal. Check DNA amount and purity. Repeat the experiment with more DNA or a new DNA prep may berequired.

7.1.4. Scoring Mutational Status

If a Cq value is Undetermined, assign a Cq of 50 and proceed to analysis. The table below should be used to determine mutational status.

Table 10. Scoring Mutational Status

Instrument	Call		QClamp® JAK2 Mutation Detection Test
Bio-Rad CFX384*	Positive:	ΔCq	≤ 13
	Negative:		> 13
Roche LightCycler® 480*	Positive:		≤ 9.8
	Negative:		> 9.8
ABI 7500*	Positive:		≤ 12.5
	Negative:		> 12.5
ABI QuantStudio 5*	Positive:		≤ 10.4
	Negative:		> 10.4

Note: * The Samples showing ΔCq values within ±1 Cq should be re-tested.

PART 8. ASSAY PERFORMANCE CHARACTERISTICS

The performance characteristics of this product were established on the Bio-Rad CFX384, ABI QuantStudio 5, Roche LightCycler® 480 qPCR instruments.

8.1. Analytical Performance

The specific performance characteristics of the QClamp® JAK2 Mutation Detection Test were determined by studies involving JAK2-defined genomic DNA reference standards from Horizon Discovery (Cambridge, England).

8.2. Cut-Offs

Along with studies for analytical accuracy the reference gDNA were tested to establish cut-offs for the assay. Cutoffs are presented in Table 10 of the manual.

8.3. Analytical Sensitivity (LOD)

The specific performance characteristics of the QClamp® JAK2 assay were determined by studies involving JAK2-defined genomic DNA reference standards and FFPE standards from cell lines with defined mutations obtained from Horizon Discovery (Cambridge, England). These samples have been characterized genetically as containing heterozygous mutations in the coding sequence of the JAK2 gene.

To determine the limit of detection (LOD) for the kit, the QClamp® assay was run using a serial dilution of mutant genomic DNA in wild-type background at different total DNA inputs and several mutation frequencies for each target. Mutant reference samples were verified by droplet digital PCR by Horizon Discovery. DNA was added to each well of the assay at 7.5, 5 and 2.5 ng/well. Mutant allele concentrations tested were 1%, 0.5%, 0.25%, and 0.1%. Results demonstrate effective clamping of wild-type, providing reproducible detection of mutations at concentrations as low as 0.1% for some assays run in multiple replicates.

Table 11. LOD Summary: Reference gDNA Samples (Bio-Rad CFX384)

DNA Input	Assay	1%	0.50%	0.25%	Wild-Type
2.5 ng	c617	100%	100%	100%	0%
5 ng	c617	100%	100%	100%	0%
7.5 ng	c617	100%	100%	100%	0%

8.4. Multiple Freeze-Thaw Cycles

The effect of 1-8 freeze-thaw cycles were tested in QClamp® JAK2 Mutation Detection Test reagents. There is no effect up to 6 freeze-thaw cycles on the QClamp® JAK2 Mutation Detection Test to distinguish between mutation positive and mutation negative samples. Caution: Repeated freeze-thaw cycles may decrease the reliability of test results.

8.4. Shelf-Life

Approximately 12 months after production of kit-see product labels for actual expiration date.

PART 9. SYMBOLS USED IN PACKAGING

Table 12. Symbols Used in Packaging

Symbol	Definition	Symbol	Definition
REF	Catalog Number	LOT	Batch Code
	Manufactured By		Expiration Date
	Temperature Limitation	EC REP	Authorized Representative in the European Community
2012-11-25	Date Format (year-month-day)		
2012-11	Date Format (year-month)		

REFERENCES

1. William Pao et al. 2005. Adenocarcinomas to Gefitinib or Erlotinib is associates with a second mutation in the JAK2 kinase domain. Plos Medicine 2(3):1-11.
2. Herbst RS, 2004. Review of epidermal growth factor receptor biology. Int. J. Radiat. Oncol. Biol. Phys. 59 (2 Suppl): 21–6.
3. Lynch TJ, Bell DW, Sordella R, et al, 2004. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N. Engl. J. Med. 350 (21): 2129–39.
4. Oda K, Matsuoka Y, Funahashi A, Kitano H, 2005. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol. Syst. Biol. 1: 2005.0010.
5. Ørum, Henrik, PCR Clamping. Current. Issues Mol. Biol. 2000; 2(1), 27-30.
6. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG and Varmus H, 2005. Acquired resistance of Lung
7. Powell et al., Detection of the hereditary hemochromatosis gene mutation by real-time fluorescence polymerase chain reaction and peptide nucleic acid clamping. Analytical Biochemistry 1998; 260: 142–8.
8. Seth D, Shaw K, Jazayeri J and Leedman PJ, 1999. Complex post-transcriptional regulation of EGF-receptor expression by EGF and TGF-α in human prostate cancer cells. Br J Cancer 80(5-6):657-69.
9. Zhang H, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, Greene MI, 2007. ErbB receptors: from oncogenes to targeted cancer therapies. J. Clin. Invest. 117 (8): 2051–8.
10. ****MIQE Reference:** "The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments". Stephen A. Bustin et. al., Clin Chem. **55** (4): 611–22 (2009). http://www.clinchem.org/content/55/4/611
11. Mycancergenome.org. Genetically informed Cancer Medicine – My Cancer Genome. [online] Available at: https://www.mycancergenome.org/.

	Product Manual QClamp® JAK2 Codon Specific Mutation Test In Codon 617 User Manual (RUO)	Document #: MAN.0010 Rev. 2

Approvals	Print Name	Signature	Date
Originator, Marketing	C. Li	[signature]	07JUN22
Ops	L. Huang		
R&D Director	M. Sha		
R&D	L. Pastor		
QA	J. Valerio		

Effective date is date of last required signature

Revision	Summary of Change	Originator	Date Initiated
0	Change Control #: 768 replaces instruction manual rev15.8R, updated doc. numbering and formatting	E. Peletskaya	27JUN16
1	Change Control #: 965 Use the same content for CE/IVD instruction manuals and RUO manuals	C. Li	15Dec17
2	Change Control #: 1418 Update product name, content and format.	C. Li	19May22

Approvals	Print Name	Signature	Date
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Ops	L. Huang	[signature]	07Jun2022
R&D Director	M. Sha	[signature]	07Jun2022
R&D	L. Pastor	[signature]	07Jun22
QA	J. Valerio	[signature]	07Jun22

Effective date is date of last required signature

Revision	Summary of Change	Originator	Date Initiated
0	Change Control #: 768 replaces instruction manual rev15.8R, updated doc. numbering and formatting	E. Peletskaya	27JUN16
1	Change Control #: 965 Use the same content for CE/IVD instruction manuals and RUO manuals	C. Li	15Dec17
2	Change Control #: 1418 Update product name, content and format.	C. Li	19May22