July 2022

ipsogen® JAK2 RGQ PCR Kit Summary of Safety and Performance

24

Version 2

IVD

For In Vitro Diagnostic Use

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

For use with *ipsogen*® JAK2 RGQ PCR Kit

CE 0197

REF 674623

QIAGEN, GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

R1 MAT 1124396

Sample to Insight

Summary of Safety and Performance

Device identification and general information	
Name or trade name, including any model number or version	*ipsogen*® JAK2 RGQ PCR Kit
Manufacturer (name and address)	QIAGEN GmbH QIAGEN Strasse 1 Hilden 40724 Germany
Basic Unique Device Identification (UDI-DI)	4053228RJAK2RGQ00000001RJ
Manufacturer's single registration number (SRN), if available	DE-MF-000004949
Medical device nomenclature	W01060299 Acquired gene or chromosome alteration tests
Class of the device	Class C

Year when the device was first placed on the EU market	The *ipsogen* JAK2 RGQ PCR Kit (24) CE (catalog number 673623, version 1), valid under EU IVD Directive 98/79/EC and Commission Decision 2010/227/EU (IVDD), was first placed on the EU market in 2014.
Authorized representative, if applicable	Not applicable
Notified body and the single identification number (SIN)	TUV Rheinland; Notified body number 0197
Intended purpose of the device	
Intended purpose	The *ipsogen* JAK2 RGQ PCR Kit is a quantitative in vitro PCR assay intended for the detection and quantification of the JAK2 V617F/G1849T mutation in genomic DNA extracted from human peripheral whole blood anticoagulated with 2K-EDTA. Results obtained with the *ipsogen* JAK2 RGQ PCR Kit are intended for use as an adjunct to evaluation of suspected Philadelphia (Ph) chromosome negative myeloproliferative neoplasm (MPN) and molecular disease monitoring in MPN patients. Any diagnostic results generated must be interpreted in conjunction with other clinical-pathological findings.
Indications and Target populations	The *ipsogen* JAK2 RGQ PCR Kit is intended for the examination of patients with suspected Philadelphia (Ph) chromosome negative myeloproliferative neoplasm (MPN) and molecular disease monitoring in MPN patients.

Contraindications and/or limitations	The kit is intended for professional use. The product is to be used only by professionals specially instructed, trained for molecular biology techniques, and familiar with the device technology. The device procedure is to be implemented in a molecular biology laboratory environment. The *ipsogen* JAK2 RGQ PCR Kit is intended to be used only with the QIAGEN Rotor-Gene® Q MDx 5plex HRM instrument and other validated workflow components as outlined in the instructions for use. The *ipsogen* JAK2 RGQ PCR Kit is not an automated device. However, the analysis is assisted by a dedicated software for automatic mutation quantification. The *ipsogen* JAK2 RGQ PCR Kit must be used following the instructions given in its Instructions for Use. Attention should be paid to expiration dates printed on the box label and the tube labels. Do not use expired components. All reagents supplied in the *ipsogen* JAK2 RGQ PCR Kit are intended to be used solely with other reagents supplied in the same kit. Failing to follow this guideline might affect performance. The *ipsogen* JAK2 RGQ PCR Kit is validated only for human peripheral whole blood anticoagulated with 2K-EDTA collected from patients with suspected or diagnosed MPN.

	The *ipsogen* JAK2 RGQ PCR Kit is validated only for use with the QIAsymphony DNA DSP Mini Kit (cat. no. 937236) or the QIAamp DSP DNA Blood Mini Kit (cat. no. 61104). The *ipsogen* JAK2 RGQ PCR Kit is validated only for use with the Rotor-Gene Q MDx 5plex HRM (for PCR) and the QIAsymphony SP (for sample preparation). Any off-label use of this product and/or modification of the components will void QIAGEN's liability. Any diagnostic results generated must be interpreted in conjunction with other clinical-pathological findings. The absence of the JAK2 V617F/G1849T mutation does not exclude the presence of other JAK2 mutations. The test can report false negative results in case of additional mutations located in nucleotides 88504 to 88622. It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance studies.
Device description	
Device description	**a) General description of the device, including its intended purpose and intended users** The *ipsogen* JAK2 RGQ PCR Kit is a quantitative in vitro PCR assay intended for the detection and quantification of the JAK2 V617F/G1849T mutation in genomic DNA extracted from human peripheral whole blood anticoagulated with 2K-EDTA.

	Results obtained with the *ipsogen* JAK2 RGQ PCR Kit are intended for use as an adjunct to evaluation of suspected Philadelphia (Ph) chromosome negative myeloproliferative neoplasm (MPN) and molecular disease monitoring in MPN patients. Any diagnostic results generated must be interpreted in conjunction with other clinical-pathological findings. The kit is intended for professional use. The product is to be used only by professionals specially instructed, trained for molecular biology techniques and familiar with the device technology. The device procedure is to be implemented in a molecular biology laboratory environment. **b) Description of the principle of the assay method or principles of operation of the instrument;** The *ipsogen* JAK2 RGQ PCR Kit exploits the qPCR oligonucleotide hydrolysis principle coupled with an Amplification Refractory Mutation System (ARMS) technique which is a simple method for detecting any mutation involving a single base change (also called Single Nucleotide Polymophism (SNP)). Polymerase Chain Reaction uses originally forward and reverse primers that hybridize to a specific DNA sequence or target sequence to amplify it. The ARMS technique is based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample. The qPCR oligonucleotide hydrolysis principle is based on a dye-linked oligonucleotide (also called probe) which is contained in the qPCR mix. This probe, which consists of an oligonucleotide labelled with a 5′ reporter dye and a downstream, 3′ dye-free quencher, hybridizes to a target sequence within the PCR product. qPCR analysis with hydrolysis

	probes exploits the 5'→3' exonuclease activity of the *Thermus aquaticus* (*Taq*) DNA polymerase. When the probe is intact, the proximity of the reporter dye to the quencher results in suppression of the reporter fluorescence primarily by Förster-type energy transfer. During PCR, if the target of interest is present, both forward and reverse primers specifically anneal and flank the probe. The 5'→3'exonuclease activity of the DNA polymerase cleaves the probe between the reporter and the quencher leading to reporter fluorescence emission. This process occurs in every cycle and does not interfere with the exponential accumulation of product. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR. In qPCR, the number of PCR cycles necessary to detect a signal above the threshold is called the Crossing point (Cp) or Cycle threshold (Ct) and is directly proportional to the amount of target present at the beginning of the reaction. **c) Rationale for qualifying the product as a device, and risk class of the device (excerpt from regulatory strategy document);** The *ipsogen* JAK2 RGQ PCR Kit is a kit of reagents intended to be used in combination with a real-time PCR instrument (QIAGEN Rotor-Gene Q MDx 5plex HRM instrument) for the examination of specimens derived from the human body for the purpose of providing information on a pathological process or state (adjunct to the evaluation and molecular response monitoring of PH(-) MPN). Thus, the product complies with the definition of an IVDMD as stated in the IVDR. The JAK2 V617F mutation is part of the diagnosis algorithm and can be also a follow-up biomarker of Myeloproliferative Neoplasms (MPN) which are Polycythemia Vera (PV), Primary Myelofibrosis (PMF) and Essential Thrombocythemia (ET). Thus the product risk class is C according to the IVDR. **d) Description of the components of the device.**

The *ipsogen* JAK2 RGQ PCR Kit contains the following components:

Material number	Component name/description	Quantity per kit in number of tubes (volume)
1073859	JAK2 MT Reaction Mix Oligonucleotides for the detection of the MT (mutant) allele and the internal control, PCR Buffer, $MgCl_2$, dNTPs *The internal amplification control included in the reaction mixes is used to monitor qPCR inhibition, and to rule out failure of the PCR reaction in case of negative results..*	1 (1010µl)
1073856	JAK2 WT Reaction Mix Oligonucleotides for the detection of the WT (wild-type) allele and the internal control, PCR Buffer, $MgCl_2$, dNTPs *The internal amplification control included in the reaction mixes is used to monitor qPCR inhibition, and to rule out failure of the PCR reaction in case of negative results.*	1 (1010µl)
1073892	*Taq DNA polymerase (HotStarTaq® 5 units/µl)*	1 (85µl)
1073865	JAK2 WT Control (100% wild-type allele) (Cell line DNA carrying 100% wild-type allele , amplification control)	1 (33µl)
1073862	JAK2 Mutant Control (100% V617F allele) (Cell line DNA carrying 100% V617F allele , amplification control)	1 (33µl)

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Material number	Component name/description	Quantity per kit in number of tubes (volume)
1095204.1 to .4 (JAK2 WT Quant Standards Set: QS1 to QS4)	JAK2 WT Quant Standard 1 (5 x 10^1 wild-type copies/5 µl)	1 (20µl)
	JAK2 WT Quant Standard 2 (5 x 10^2 wild-type copies/5 µl)	1 (20µl)
	JAK2 WT Quant Standard 3 (5 x 10^3 wild-type copies/5 µl)	1 (20µl)
	JAK2 WT Quant Standard 4 (5 x 10^4 wild-type copies/5 µl)	1 (20µl)
1095205.1 to .4 (JAK2 MT Quant Standards Set: QS1 to QS4)	JAK2 MT Quant Standard 1 (5 x 10^1 V617F copies/5 µl)	1 (20µl)
	JAK2 MT Quant Standard 2 (5 x 10^2 V617F copies/5 µl)	1 (20µl)
	JAK2 MT Quant Standard 3 (5 x 10^3 V617F copies/5 µl)	1 (20µl)
	JAK2 MT Quant Standard 4 (5 x 10^4 V617F copies/5 µl)	1 (20µl)
1067627	Water for no template control (NTC) (nuclease-free water)	1 (1.9ml)
1073894	TE (Tris EDTA) buffer for sample dilution	1 (1.9ml)

The JAK2 MT Quant Standards (QS1 to QS4) are serial dilutions of plasmids carrying the V617F allele sequence.
The JAK2 WT Quant Standards (QS1 to QS4) are serial dilutions of plasmids carrying the WT allele sequence.

e) The description of the specimen collection and transport materials provided with the device;

No specimen collection nor transport materials are provided with the device.

f) For instruments of automated assays: the description of the appropriate assay characteristics or dedicated assays;

	The *ipsogen* JAK2 RGQ PCR Kit is not an automated assay. However, the analysis is assisted by a dedicated software suite. **g) For automated assays: a description of the appropriate instrumentation characteristics or dedicated instrumentation;** The *ipsogen* JAK2 RGQ PCR Kit is not an automated assay. However, the analysis is assisted by a dedicated software suite. **h) A description of any software to be used with the device;** The *ipsogen* JAK2 RGQ PCR Kit is intended to be used only with the QIAGEN Rotor-Gene Q MDx 5plex HRM instrument and other validated workflow components as outlined in the instructions for use. The *ipsogen* JAK2 RGQ PCR Kit is not an automated device. However, the analysis is assisted by a dedicated software: the Rotor-Gene AssayManager® software version 2.1.x (x≥0), with the Rotor-Gene AssayManager Gamma Plug-in version 1.0.x (x≥0) and the ipsogen _JAK2_blodd_CE_IVDR Assay Profile (AP_ipsogen_JAK2_blood_CE_IVDR_V2_0_x.iap (x≥1)). **i) A description or complete list of the various configurations or variants of the device that are intended to be made available in the market;** There is currently no variant of the *ipsogen* JAK2 RGQ PCR Kit (674623) that is planned to be commercialized in the European Union. **j) A description of the accessories for the device, other devices, and other products that are not devices, which are intended to be used in combination with the device.**

	There is currently no accessory designed for the *ipsogen* JAK2 RGQ PCR Kit. This kit is a ready to use set of reagents. Users of the *ipsogen* JAK2 RGQ PCR Kit will have to provide the following equipment and materials required but not provided with the Kit to perform the whole workflow: ● Consumables and reagents for manual DNA extraction ○ QIAamp® DSP DNA Blood Mini Kit (cat. no. 61104) ○ Ethanol (96–100%) **Note**: Do not use denatured alcohol as this contains other substances such as methanol or methylethylketone. ● Consumables and reagents for automated DNA extraction ○ QIAsymphony DSP DNA Mini Kit (cat. no. 937236) ○ Sample Prep Cartridges, 8-well (cat. no. 997002) ○ 8-Rod Covers (cat. no. 997004) ○ Filter-Tips, 1500 µl (cat. no. 997024) ○ Filter-Tips, 200 µl (cat. no. 990332) ○ Elution Microtubes CL (cat. no. 19588) ○ Tip disposal bags (cat. no. 9013395) ○ Micro tubes 2.0 ml Type H (Sarstedt®, cat. no. 72.694, **www.sarstedt.com**) ● Consumables and reagents for PCR ○ Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters ○ 1.5 ml or 2.0 ml nuclease-free PCR tubes

	○ Strip Tubes and Caps, 0.1 ml, for the Rotor-Gene Q (cat. no. 981103 or 981106) ○ Ice ● Equipment ○ Adjustable pipettes* dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl) ○ Disposable gloves ○ Vortex mixer ○ Heating block for lysis of samples at 56°C ○ Benchtop centrifuge* with rotor for 0.5/1.5/2.0 ml reaction tubes (capable of attaining 13,000–14,000 rpm) ○ Spectrophotometer* ● Equipment for sample preparation ○ QIAsymphony SP instrument* (cat. no. 9001297), software version 4.0 or later, provided accessories, and Blood_200_V7_DSP protocol (or later version) ○ Tube Insert 3B (Insert, 2.0 ml v2, sample carrier (samplecarr.) (24), Qsym, cat. no. 9242083) ● Equipment for real-time PCR ○ Real-time PCR instrument*: Rotor-Gene Q MDx 5plex HRM Platform (cat. no. 9002032) or Rotor-Gene Q MDx 5plex HRM System (cat. no. 9002033) and provided accessories ○ Installed Rotor-Gene AssayManager® software version 2.1.x (x≥0)

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

	○ Installed Rotor-Gene AssayManager Gamma Plug-in version 1.0.x (x≥0) ○ Imported ipsogen _JAK2_blood_CE_IVDR Assay Profile (AP_ipsogen_JAK2_blood_CE_IVDR_V2_2_x.iap (x≥1))
Reference to previous generation(s) or variants of the device (as applicable) and a description of the differences	The *ipsogen* JAK2 RGQ PCR Kit (24) CE (catalog number 673623, version 1), valid under EU IVD Directive 98/79/EC and Commission Decision 2010/227/EU (IVDD), was first placed on the EU market in 2014. The *ipsogen* JAK2 RGQ PCR Kit (catalog number 674623) is a Version 2 that has undergone a program to ensure compliance with the In Vitro Diagnostic Devices Regulation EU/2017/746 (IVDR). For both kits 673623 and 674623, the kit components are identical and both kits are technically identical with an analysis assisted by a software for automatic mutation quantification. An assay profile dedicated to *ipsogen* JAK2 RGQ PCR Kit catalog number 674623 has been generated based on the existing version dedicated to *ipsogen*® JAK2 RGQ PCR Kit (24) CE catalog number 673623. Compared to the instructions for use of the *ipsogen* JAK2 RGQ PCR Kit (24) CE (catalog number 673623), the instructions for use of the *ipsogen* JAK2 RGQ PCR Kit (catalog number 674623) has the below listed improvements to comply with IVDR requirements: ● Intended Use and Intended User statement detailed and specified ● Protocols have been re-arranged and supplemented with additional instructions and illustrations to improve understanding ● Genomic DNA stability and in-use stability claims have been updated

	• Performance Characteristics have been updated and supplemented with additional data (analytical and clinical) • Reference to the summary of safety and performance resource has been added Symbols have been updated and supplemented with additional labels.
Description of accessories intended to be used in combination with the device (as applicable)	Not applicable.
Description of other devices and products intended to be used in combination with the device (as applicable)	There is currently no accessory designed for the *ipsogen* JAK2 RGQ PCR Kit. This kit is a ready to use set of reagents. Users of the *ipsogen* JAK2 RGQ PCR Kit will have to provide the following equipment and materials required but not provided with the Kit to perform the whole workflow: • Consumables and reagents for manual DNA extraction ○ QIAamp DSP DNA Blood Mini Kit (cat. no. 61104) ○ Ethanol (96–100%) **Note**: Do not use denatured alcohol as this contains other substances such as methanol or methylethylketone. • Consumables and reagents for automated DNA extraction ○ QIAsymphony DSP DNA Mini Kit (cat. no. 937236)

	○ Sample Prep Cartridges, 8-well (cat. no. 997002) ○ 8-Rod Covers (cat. no. 997004) ○ Filter-Tips, 1500 µl (cat. no. 997024) ○ Filter-Tips, 200 µl (cat. no. 990332) ○ Elution Microtubes CL (cat. no. 19588) ○ Tip disposal bags (cat. no. 9013395) ○ Micro tubes 2.0 ml Type H (Sarstedt, cat. no. 72.694, **www.sarstedt.com**) ● Consumables and reagents for PCR ○ Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters ○ 1.5 ml or 2.0 ml nuclease-free PCR tubes ○ Strip Tubes and Caps, 0.1 ml, for the Rotor-Gene Q (cat. no. 981103 or 981106) ○ Ice ● Equipment ○ Adjustable pipettes* dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl) ○ Disposable gloves ○ Vortex mixer ○ Heating block for lysis of samples at 56°C ○ Benchtop centrifuge* with rotor for 0.5/1.5/2.0 ml reaction tubes (capable of attaining 13,000–14,000 rpm) ○ Spectrophotometer*

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

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Equipment for sample preparation

QIAsymphony SP instrument* (cat. no. 9001297), software version 4.0 or later, provided accessories, and Blood_200_V7_DSP protocol (or later version)
Tube Insert 3B (Insert, 2.0 ml v2, sample carrier (samplecarr.) (24), Qsym, cat. no. 9242083)

Equipment for real-time PCR

Real-time PCR instrument*: Rotor-Gene Q MDx 5plex HRM Platform (cat. no. 9002032) or Rotor-Gene Q MDx 5plex HRM System (cat. no. 9002033) and provided accessories
Installed Rotor-Gene AssayManager software version 2.1.x (x≥0)
Installed Rotor-Gene AssayManager Gamma Plug-in version 1.0.x (x≥0)
Imported ipsogen_JAK2_blood_CE_IVDR Assay Profile (AP_ipsogen_JAK2_blood_CE_IVDR_V2_2_x.iap (x≥1))

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<tr><td colspan="2">Standards reference</td></tr>
<tr><td>Harmonized standards and Common Specifications (CS) applied</td><td>
There are no harmonized standards under IVDR. The table below shows the standards used for the ipsogen JAK2 RGQ PCR Kit development.
<table>
<tr><th>Standard name</th><th>Title of standard</th></tr>
<tr><td>EN ISO 13485:2016</td><td>Medical devices - Quality management systems - Requirements for regulatory purposes (ISO 13485:2016)</td></tr>
<tr><td>EN ISO 14971:2019</td><td>Medical devices - Application of risk to medical devices</td></tr>
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* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

	EN ISO 15223-1:2016	Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements
	EN ISO 18113-1:2011	In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements
	EN ISO 18113-2:2011	In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use
	EN ISO 23640:2015	In vitro diagnostic medical devices - Evaluation of stability of in vitro diagnostic reagents (ISO 23640:2011)
	EN 62304:2006	Medical device software - Software life-cycle processes (IEC 62304:2006)
	EN 62366:2008	Medical devices - Application of usability engineering to medical devices (IEC 62366:2007)

Summary of the performance evaluation and post-market performance follow-up

Summary of the performance evaluation and post-market performance follow-up	The performance evaluation verifies the scientific validity, analytical performance and, where appropriate, the clinical performance of the *ipsogen* JAK2 RGQ PCR Kit to allow a structured and transparent process that generates reliable data and robust studies. Scientific validity assessment was based on a systematic literature review, assessment of available/ retrieved/ new data relevant to the *ipsogen* JAK2 RGQ PCR Kit and its intended purpose and consensus experts opinions/positions from international guidelines. The herewith presented data demonstrate the scientific validity of the *ipsogen* JAK2 RGQ PCR Kit for its Intended Use. Analytical performance was demonstrated based on the investigations achieving the requested performance indicators: Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ), precision (repeatability and reproducibility), linearity, interfering

	substances, cross-contamination, PCR accuracy and testing of WHO JAK2 panel 16/120 (concordance, trueness, and accuracy), measuring range, specimen stability/handling, acceptance criteria for DNA quantification, comparison of manual and automatic extraction, usability verification, use of contrived samples, and assay profile verification. The assessment of these sources showed that the analytical performance of the *ipsogen* JAK2 RGQ PCR Kit is adequate for its Intended Use and ensures a safe use for the intended purpose, user, and patient population. Clinical performance was demonstrated based on a systematic literature review, clinical performance studies demonstrating clinical performance indicators for accuracy/concordance: PPV, NPV, diagnostic sensitivity, specificity, likelihood ratio using the Clinical Performance study data and PPA and NPA using the Analytical Accuracy study and Clinical Performance study data. Also, experience gained by routine diagnostic testing was evaluated. The assessment of these sources showed that the clinical performance of the *ipsogen* JAK2 RGQ PCR Kit is adequate for its Intended Use and ensures a safe use for the intended purpose, user, and patient population. The Post Market evaluation system aims to confirm the safety, performance, and scientific validity of the *ipsogen* JAK2 RGQ PCR Kit throughout its expected lifetime, to ensure the continued acceptability of the benefit/ risk ratio, and to detect emerging risks on the basis of factual evidence, and determine, apply, and evaluate any preventive and corrective action. The Post-market Performance Follow-up aims to confirm the safety, performance and scientific validity of the *ipsogen* JAK2 RGQ PCR Kit throughout the expected lifetime of the device, to ensure the continued

	acceptability of the benefit/ risk ratio and to detect emerging risks on the basis of factual evidence. The purpose is to verify clinical safety and performance over expected lifetime, to identify previously unknown risks or limits to performances and contra-indications, to identify and to analyze emergent risks on the basis of factual evidence, to ensure continuous acceptability of the clinical evidence and the benefit-risk ratio, and identify possible systematic misuse, the following post-market performance follow up data will be collected. The clinical experience gained related to trends will be gathered from data generated from post-market studies (company-sponsored or investigator-initiated), patient registers Real World Data and Evidence (RWD/RWE), where applicable. Feedback from users (health care professionals, clinical KOLs, and lab professionals), and distributors and importers from surveys, and published data on user perspectives, and sales and training. Scientific literature evaluation after a literature search for the device and similar and equivalent devices, new technical or medical guidelines. Information on technical or specialized Records, Registers, case reports reviewed and evaluated by QIAGEN. Epidemiological studies as observational post-marketing studies to collect information about the performance of the device. The Post-market Performance Follow-up will be updated each year to integrate new data and results, post-market studies, references to

relevant Common Specifications harmonized standards consulted and relevant post-market performance-follow up guidance.

Pre-specified results can trigger additional tasks and activities. Pre-specified triggers for post-market performance-follow up activities are based on their impact on product claims and benefit-risk and can include customer complaints, emergence of data from publications, external quality assessment programs, and other registries.

Summary of analytical performance

Limit of blank

Limit of blank (LOB) was determined following the CLSI/NCCLS EP17-A2 standard, on 30 healthy donor whole blood samples with a wild-type (WT) JAK2 status, using three reagent lots (120 measurements/lot).

Summary of the LOB results

	Measured LOB	Final limit of blank
Lot 1	0%	
Lot 2	0%	0%
Lot 3	0%	

This corresponds to the expected value in a normal population using the *ipsogen* JAK2 RGQ PCR Kit.

Limit of detection

Limit of detection (LOD or analytical sensitivity) was determined based on the "Probit approach" described in the CLSI/NCCLS EP17-A2 standard. In this study, 6 low-levels of mutation were analyzed for 3 independent samples (MPN whole blood DNA spiked into wild-type

(WT) whole blood DNA), with 3 lots, 60 measurements per sample and per mutation. The results obtained indicated the analytical sensitivity was 0.042% of JAK2 V617F mutation.

Summary of the LOD results

	Measured LOD	Final limit of detection
Lot 1	0.041%	
Lot 2	0.029%	0.042%
Lot 3	0.042%	

Limit of quantitation

Limit of quantitation (LOQ) definition and determination was based on the CLSI/NCCLS EP17-A2 guideline. LoQ was defined as the lowest JAK2 V617F mutation percentage level that can be accurately distinguished from the *ipsogen* JAK2 RGQ PCR Kit's LoD with a confidence interval of 95% (risk $\alpha = 0.05$). Data from the single-site repeatability study were used to calculate the *ipsogen* JAK2 RGQ PCR Kit's LoQ. The results obtained indicate the LoQ is 0.233% of JAK2 V617F mutation.

In the context of molecular disease monitoring, this implies that if the measured JAK2 V617F mutation percentage is below 0.233% at a given point in time, a JAK2 V617F allele burden reduction cannot be reliably quantified at the next time point.

Linearity

The linearity of the quantification of the JAK2 mutation in MPN patients was assessed according to the CLSI/NCCLS EP06AE standard, with one lot of *ipsogen* JAK2 RGQ PCR Kit and with testing on 11 levels of

mutation for five different DNA inputs. The quantification of the JAK2 mutation burden in MPN samples is linear; i.e., the *ipsogen* JAK2 RGQ PCR Kit is able to quantify samples from the LOD value to 100% mutation, which corresponds to the expected values in the affected population, as long as the quantified sample concentration is close to 10 ng/µl (between 5 and 20 ng/µl).

Repeatability and reproducibility

The single-site precision study design meets the requirements of the CLSI/NCCLS EP5-A3 standard. Testing was performed on 11 levels of mutation, from 0.07% to 72.67%, using serial dilutions of a clinical sample from a MPN patient. For each level of mutation, 108 measurements were obtained by three operators over 27 days (two replicates per run and two runs per day) using three lots of *ipsogen* JAK2 RGQ PCR Kit and three Rotor-Gene Q MDx 5plex HRM instruments. The precision for the 100% level is expressed by comparison to the precision determined for the 72.67% level, based on trend analyses supported by additional data obtained on a 100% JAK2 V617F sample consisting of DNA from the MUTZ-8 cell line (38 measurements).

Precision results: repeatability (single-site study)

Sample	Mean JAK2 mutation percentage	SD_{R+}	SD_{RUN++}	$SD_{TOTAL+++}$	CV_{TOTAL}
S0	100	ND	ND	≤ 5.45	≤ 7.50%
S1	72.67	1.99	2.99	5.45	7.50%
S2	53.96	2.48	3.16	6.52	12.09%
S3	23.13	1.59	1.95	4.51	19.52%
S4	11.97	1.10	1.17	2.79	23.27%
S5	6.01	0.71	0.63	1.57	26.17%

S6	2.39	0.31	0.36	0.70	29.23%
S7	1.23	0.17	0.16	0.34	27.38%
S8	0.63	0.13	0.12	0.24	37.88%
S9	0.13	0.05	0.03	0.07	52.31%
S10	0.07	0.03	0.02	0.04	65.01%

SD: Standard deviation
R+: Repeatability.
RUN++: Between run precision.
TOTAL+++: Total precision (including inter-instrument, inter-operator and inter-lot).
CV_{TOTAL}: Coefficient of variation for the total precision in percentage.
ND: not determined

The inter-laboratory precision study design meets the requirements of the CLSI/NCCLS EP5-A3 standard. The study involved four sites (France, Germany, and two sites in the USA). Testing was performed on seven levels of mutation, from 1.21% to 67.64%, using dilutions of the MUTZ-8 cell line in healthy donor whole blood (i.e. contrived samples). Each site performed three DNA extraction runs using the QIAsymphony SP instrument and a unique batch of the QIAsymphony DSP DNA Mini Kit. Each DNA extract was tested in eight qPCR runs (two runs per day and per site over four non-consecutive days) using a unique batch of *ipsogen* JAK2 RGQ PCR Kit, giving rise to 96 expected measurements per sample over all sites.

The L2 sample was invalid in one extraction run leading to a total number of 88 qPCR tests instead of 96. In addition, one qPCR run was invalid, leading to three invalid tests for all samples (except L2, i.e. 2 invalid results). Moreover, the L7 sample was invalid in one qPCR run and L4 was invalid in two qPCR runs leading to two additional invalid tests.

The precision for the 100% level is expressed by comparison to the precision determined for the 67.64% level, based on trend analyses supported by additional data obtained on a 100% JAK2 V617F sample consisting of DNA from the MUTZ-8 cell line (38 measurements).

Precision results: reproducibility (inter-laboratory study)

Sample	Total tests	Total invalid tests	JAK2%MT mean	Within run, SD, %CV	Between run within day, SD, %CV	Between day, SD, %CV	Between site, SD, %CV	Total, SD, %CV
L0	N/A	N/A	100	N/A	N/A	N/A	N/A	≤ 4.074, ≤ 6.02
L1	96	3	67.64	2.616, 3.87	2.060, 3.05	1.999, 2.96	1.530, 2.26	4.074, 6.02
L2	88	2	40.03	3.482, 8.70	1.011, 2.53	2.389, 5.97	0.986, 2.46	4.387, 10.96
L3	96	3	22.26	3.318, 14.90	1.256, 5.64	1.257, 5.64	0.803, 3.61	3.807, 17.10
L4	96	5	8.02	1.770, 22.06	0.516, 6.44	0.000, 0.00	0.000, 0.00	1.841, 22.95
L5	96	3	4.35	0.706, 6.23	0.547, 12.57	0.000, 0.00	0.197, 4.53	0.906, 20.82
L6	96	3	2.03	0.246, 12.15	0.365, 18.00	0.063, 3.11	0.000, 0.00	0.441, 21.76
L7	96	4	1.21	0.104, 8.62	0.057, 4.72	0.211, 17.43	0.000, 0.00	0.189, 15.64

JAK2%MT: JAK2 mutation percentage; **SD**: Standard deviation; **CV**: coefficient of variation in percentage; **N/A**: Not applicable.

An additional inter-laboratory study was conducted across three test sites (one in Europe and two in the USA), on four whole blood samples from MPN patients (i.e. clinical samples). Each site performed three DNA extraction runs. Each DNA extract was tested in 12 qPCR runs (one replicate per run per sample, two runs per day per operator at each site - two operators per site were involved - over three non-consecutive days)

on one Rotor-Gene Q MDx instrument using a single lot of *ipsogen* JAK2 RGQ PCR Kit. For each sample, 36 measurements were obtained.

Additional inter-laboratory study results

Sample	N	JAK2%MT mean	Within run, SD, %CV	Between run within day, SD, %CV	Between day, SD, %CV	Between site, SD, %CV	Total, SD, %CV
Sample 1	36	95.19	0.995, 1.04	0.000, 0.00	0.541, 0.57	0.000, 0.00	1.130, 1.19
Sample 2	36	22.83	3.988, 17.47	0.000, 0.00	1.707, 7.48	1.552, 6.80	4.501, 19.72
Sample 3	36	14.44	2.257, 15.63	1.398, 9.68	0.000, 0.00	1.422, 9.84	2.890, 20.01
Sample 4	36	4.03	0.186, 4.63	0.835, 20.74	0.000, 0.00	0.608, 15.09	0.922, 22.91

JAK2% MT Mean: JAK2 mutation percentage; **N**: Number of measurements, **SD**: Standard deviation; **CV**: coefficient of variation in percentage.

Interfering substances

The study design meets the requirements of the NCCLS standard EP7-A3 "Interference Testing in clinical Chemistry". A total of 19 substances potentially present in blood samples were chosen for their potential effect on PCR (busulfan, citalopram hydrobromide, paroxetine hydrochloride hemihydrate, sertraline hydrochloride, fluoxetine hydrochloride, acetaminophen [paracetamol], bilirubin unconjugated, potassium 2K EDTA and 3K EDTA, sodium EDTA, Hgb [human], triglycerides, lisinopril dehydrate, hydroxyurea, acetylsalicylic acid, salicylic acid, thiotepa, anagrelide, interferon alpha 2b).

Substances from the DNA extraction process were also assessed (QSL1, QSB1, QSW1, QSW2 and PK from the QIAsymphony DSP DNA Blood

Mini Kit; QIAGEN Protease, ethanol, AW1and AW2 from the QIAamp DSP DNA Blood Mini Kit).

The obtained results showed no interfering effect for these substances.

Interfering substances

Tested substance	Tested concentration
Bilirubin unconjugated	150.3 µg/mL
Hemoglobin [human]	2000 µg/mL
Triglycerides	30000 µg/mL
Busulfan	38.4 µg/mL
Citalopram hydrobromide	0.75 µg/mL
Paroxetine hydrochloride hemihydrate	1.14 µg/mL
Sertraline hydrochloride	0.67 µg/mL
Fluoxetine hydrochloride	3.87 µg/mL
Acetaminophen [paracetamol]	200.7 µg/mL
Lisinopril dehydrate	0.33 µg/mL
Hydroxyurea	28.2 µg/mL
Acetylsalicylic acid	651.6 µg/mL

Tested substance	Tested concentration
Salicylic acid	0.6 µg/mL
Thio-tepa	48 µg/mL
Anagrelide	6 µg/mL
Interferon alpha 2b*	1.8 MU/L
Potassium EDTA (2K-EDTA)	2X (3600 µg/mL)
Potassium EDTA (3K-EDTA) **	1X (1800 µg/mL), 3X (5400 µg/ml)
Sodium EDTA (2Na-EDTA) **	1X (3000 µg/ml), 3X (9000 µg/ml)
QSL1	2% of total sample volume

QSB1	2% of total sample volume
QSW1	2% of total sample volume
QSW2	2% of total sample volume
Proteinase K (PK) †	2% of total sample volume
Proteinase K (PK) †	2x the expected remaining volume after the extraction process 3x the expected remaining volume after the extraction process
QIAGEN Protease	1,29E-05 % of total sample volume
Ethanol (EtOH)	1,29E-03 % of total sample volume
Buffer AW1	1,00 E-01 % of total sample volume
Buffer AW2	1,00 % of total sample volume

* The recommended dosage for PV patients is 3 MU, which is assumed to be distributed in 5L of blood (80Kg individual), resulting in a concentration of 0.6 MU/L. Following recommendations from the NCCLS standard EP7-A2, three times this concentration was tested, i.e. 1.8 MU/L.

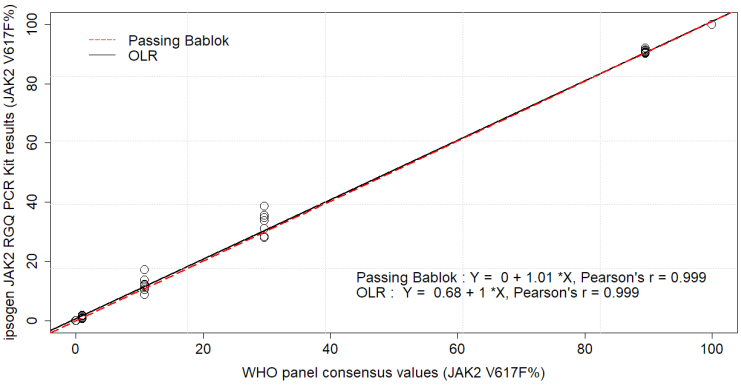
** 1X concentration according to provider

† PK causes an interfering effect when tested at 2% of the total sample volume (unlikely to occur); further testing confirmed PK is removed during the extraction process: no interference is expected under normal use conditions.

Testing of WHO International Reference Panel for Genomic JAK2 V617F (NIBSC, panel code 16/120)

The WHO 1st International Reference Panel for Genomic JAK2 V617F developed by the National Institute for Biological Standards and Control (NIBSC, panel code 16/120) was tested using three lots of the ipsogen JAK2 RGQ PCR Kit (three replicates per level of the reference panel and per reagent lot). The experiments were performed over three days by one operator, using one Rotor-Gene Q 5plex HRM instrument. The concordance between the ipsogen JAK2 RGQ PCR Kit results and the consensus values published in the Reference Panel's Instructions for Use was assessed using an ordinary linear regression (slope: 1.003, 95%CI [0.997 ; 1.010] – intercept: 0.677, 95%CI [0.212 ; 1.289]) and a

Passing-Bablok regression (slope: 1.01, 95%CI [1.00 ; 1.021] – intercept: 0.00, 95%CI [-0.02 ; 0.010]). Concordance is confirmed, demonstrating the suitability of the kit in providing JAK2 V617F data which are in agreement with other commonly used diagnostic techniques.



Concordance between the ipsogen JAK2 RGQ PCR Kit results and the WHO International Reference Panel for Genomic JAK2 V617F (NIBSC, panel code 16/120) consensus values.

Concordance was assessed using an ordinary Linear Regression (OLR) and a Passing Bablok Regression.

The panel comprises seven JAK2 V617F levels: 100%, 89.5%, 29.6%, 10.8%, 1.00%, 0.03% and 0%. The WHO consensus values were determined using a range of commonly used techniques as part of an international collaborative study; the reference values attributed to each JAK2 V617F% level are median values (more information on **https://www.nibsc.org**).

Trueness and accuracy

Measurement trueness is inversely related to the systematic measurement error (SE, or bias). Bias was calculated based on instructions from the NCCLS guideline EP09c, for each JAK2 V617F% level of the reference panel, for each reagent lot as well as overall reagent lots, using data from the above-described study. The highest bias values were obtained with the *ipsogen* JAK2 RGQ PCR Kit lot 2.

The accuracy is the closeness of agreement between a test result and the accepted reference value (in this case, the value assigned to each JAK2 V617F% level of the WHO panel). Accuracy takes into account both trueness and precision, and is inversely proportional to the total error, calculated as shown in table below.

Bias and measurement error

WHO panel *Ampoule code* **Reference value**	***ipsogen* JAK2 RGQ PCR Kit lot**	**Bias (SE) Per lot** *[95% CI]*	**Bias (SE) Overall** *[95% CI]*	**Total error (Accuracy)**
15/172 0%	1	0.000 [N/A]	0.001 [-0.001 ; 0.004]	0.010
	2	0.003 [-0.011 ; 0.018]		
	3	0.000 [N/A]		
15/170 0.03 %	1	-0.010 [-0.053 ; 0.033]	0.003 [-0.021 ; 0.028]	0.024
	2	0.020 [-0.094 ; 0.134]		

		3	0.000 [-0.075 ; 0.075]		
	15/168 1.00 %	1	-0.310 [-0.621 ; 0.001]	0.066 [-0.276 ; 0.407]	0.363
		2	0.617 [0.016 ; 1.217]		
		3	-0.110 [-0.261 ; 0.041]		
	15/166 10.8 %	1	-0.183 [-4.523 ; 4.156]	1.207 [-0.630 ; 3.043]	2.521
		2	3.600 [-2.670 ; 9.870]		
		3	0.203 [-1.387 ; 1.793]		
	15/244 29.6 %	1	0.970 [-8.238 ; 10.178]	2.874 [0.016 ; 5.733]	5.589
		2	6.347 [0.141 ; 12.552]		
		3	1.307 [-5.767 ; 8.381]		
	15/246 89.5 %	1	1.000 [-0.295 ; 2.295]	1.381 [0.889 ; 1.873]	≤ 5.622
		2	1.783 [-0.316 ; 3.883]		

	3	1.360 [0.270 ; 2.450]		
15/164 100%	1	-0.017 [-0.031 ; -0.002]	-0.017 [-0.021; -0.013]	≤ 5.450
	2	-0.020 *[N/A]*		
	3	-0.013 *[-0.028 ; 0.001]*		

SE: systematic error or bias, i.e. the difference between the average of individual measurements obtained with the *ipsogen* JAK2 RGQ PCR Kit ($\overline{V}_{JAK2\ Kit}$) and the WHO reference panel consensus value (V_{Ref}).

$$SE\ (\%) = \frac{\overline{V}_{JAK2\ Kit} - V_{Ref}}{V_{Ref}} \times 100$$

The total error (TE) is calculated as $TE = \sqrt{s^2 + SE^2}$, where s is the standard deviation (random error).

95% CI: 95% confidence interval

N/A: not applicable

Analytical accuracy

The purpose of this study was to validate the analytical accuracy of the *ipsogen* JAK2 RGQ PCR Kit under conditions of normal use with clinical samples from subjects suspected of having myeloproliferative neoplasms. This study was performed on gDNA samples extracted from a total of 473 specimens: 276 with suspected PV, 98 with ET and 99 with PMF. The JAK2 V617F status of the patient samples obtained with the *ipsogen* JAK2 RGQ PCR Kit was compared with the JAK2 V617F status obtained with the reference method for JAK2 status determination, i.e., an independently validated bi-directional sequencing (BDS). As the *ipsogen* JAK2 RGQ PCR Kit's LoD is 0.042% of JAK2 V617F, the JAK2 V617F status of a patient sample tested with the ipsogen JAK2 RGQ PCR Kit is positive above or at this limit and negative below this limit.

Of the 473 specimens, 22 specimens were JAK2-positive with the *ipsogen* JAK2 RGQ PCR Kit while negative with BDS.

The overall agreement is 95.35% (451/473 subjects; 95% CI: 93.04%, 97.06%). The positive agreement was 100% (165/165 subjects; 95% CI: 97.79%, 100%) and the negative agreement was 92.86 % (286/308 subjects; 95% CI: 89.39%; 95.47%). The results are shown below.

Concordance between the *ipsogen* JAK2 RGQ PCR Kit and Sanger bi-directional sequencing in MPN population (combined ET, PMF and PV populations)

		Sanger Bi-directional sequencing		
		JAK2 V617F positive	**JAK2 V617F negative**	**Total**
***ipsogen* JAK2 RGQ PCR kit**	**JAK2 V617F positive**	165	22	187
	JAK2 V617F negative	0	286	286
	Total	**165**	**308**	**473**

Assessment of analytical accuracy study results in MPN cohorts

The concordance between results obtained for the JAK2 V617F mutation with the *ipsogen* JAK2 RGQ PCR Kit and with Sanger sequencing (BDS) in subjects with ET, PMF, and PV are provided separately:

- For ET, the overall agreement is 89.8% (88/98 subjects; 95% CI: 82.03–95.0%), the positive agreement is 100% (43/43 subjects; 95% CI: 91.78–100%) and the negative agreement is 81.82% (45/55 subjects; 95% CI: 69.1–90.92%).

	• For PMF, the overall agreement is 93.94% (93/99 subjects; 95% CI: 87.27–97.74%), the positive agreement is 100% (51/51 subjects; 95% CI: 93.02–100%) and the negative agreement is 87.5% (42/48 subjects; 95% CI: 74.75–95.27%). • For PV, the overall agreement is 97.83% (270/276 subjects; 95% CI: 95.33–99.2%), the positive agreement is 100% (71/71 subjects; 95% CI: 94.94–100%) and the negative agreement is 97.07% (199/205 subjects; 95% CI: 93.74–98.92%). The specimens yielding discordant results appeared to have mutation levels below the BDS detection capability (around 10%). Because Sanger sequencing is not as sensitive as the *ipsogen* JAK2 RGQ PCR Kit which can report values down to 0.042% of JAK2 V617F (i.e the LoD value), a separate study was conducted using a validated next-generation sequencing (NGS) method to detect JAK2 V617F allele in the 15/22 discordant samples (nine ET, five PMF and one PV), as well as a randomly selected set of 22 JAK2 V617F-positive and -negative concordant specimens. The JAK2 V617F status of patient samples was determined by the NGS method based on its limit of analytical sensitivity (i.e between 1% and 2% of JAK2 V617F). Therefore, the JAK2 V617F status of a patient sample was positive if the JAK2 V617F mutation was detected by the NGS method and, reciprocally the JAK2 V617F status was negative if the JAK2 V617F mutation was not detected. All 15 discordant specimens tested positive by NGS, agreeing with the *ipsogen* JAK2 RGQ PCR Kit. All concordant samples tested the same with NGS and in agreement with the *ipsogen* JAK2 RGQ PCR Kit and BDS. The 7 other samples were considered as discordant as NGS data are not available for these samples. Conclusion of the analytical accuracy study.

	The *ipsogen* JAK2 RGQ PCR Kit was 98.3% accurate for the detection of JAK2 V617F allele in specimens from subjects with JAK2 V617F levels ≥0.042% (i.e the LoD value).
Summary of clinical performance	Sensitivity was 94.64% (95% CI; 85.13%, 98.88%) indicating that the *ipsogen* JAK2 RGQ PCR Kit within the WHO diagnostic criteria is expected to detect PV in the vast majority of subjects with the disease. Specificity of PV diagnosis using the *ipsogen* JAK2 RGQ PCR Kit within the WHO diagnostic criteria was 95.62% (95% CI; 91.19%, 98.22%), indicating that it is also expected to rule out PV in the vast majority of subjects without PV. Using the *ipsogen* JAK2 RGQ PCR Kit within the WHO diagnostic criteria PPV was 88.33% (95% CI; 77.27%, 93.57%)* and NPV was 98.08% (95% CI; 94.8%, 99.4%). The likelihood ratio of a negative test using the *ipsogen* JAK2 RGQ PCR Kit, for the PV diagnosis, within the WHO diagnostic criteria was 21.61 (95% CI; 10.44, 44.71), indicating that the JAK2 V617F positive result is more likely to occur in subjects with PV than in those without PV. The likelihood ratio of a positive test using the *ipsogen* JAK2 RGQ PCR Kit, for the PV diagnosis, within the WHO diagnostic criteria was 0.06 (95% CI; 0.02, 0.18), indicating that the JAK2 V617F negative result is much less likely to occur in subjects with PV than in those without PV.

* PPV is dependent on prevalence. Due to the prevalence being low in the study population; and sensitivity and specificity are independent of prevalence, sensitivity and specificity are more relevant.

Metrological traceability	
Metrological traceability of assigned values	The metrological traceability of values assigned to calibrators and control materials, including identification of applied reference materials, and/or reference measurement procedures of higher order, and information regarding maximum (self-allowed) batch-to-batch variation provided with relevant figures and units of measure. The WHO 1st International Reference Panel for Genomic JAK2 V617F (NIBSC panel code 16/120) was established in 2016 by the Expert Committee on Biological Standardization of the World Health Organization (WHO) (see WHO document WHO/BS/2016.2293). The panel comprises seven freeze-dried human genomic DNA materials produced by the combining of genomic DNA derived from JAK2 wild-type and JAK2 V617F cell lines, providing standards at a range of clinically relevant JAK2 V617F levels, expressed as a percentage of total JAK2, from 0% to 100%. See **www.nibsc.org/science_and_research/advanced therapies/genomic_reference_materials/jak_2_v617f_(who).aspx** and Sanzone AP et al. (2016) *Collaborative study to evaluate the proposed WHO 1st International Reference Panel for Genomic JAK2 V617F*. Neither this panel nor value assigned calibrators (derived from this standard material) are included into the *ipsogen*® JAK2 RGQ PCR Kit. The *ipsogen* JAK2 RGQ PCR Kit does contain control materials, but these are not derived from the WHO reference material. Therefore, there is no metrological traceability report.

	Still, the concordance of the *ipsogen* JAK2 RGQ PCR Kit results with the panel's consensus values has been assessed and confirmed: This study is described in *ipsogen JAK2 RGQ PCR Kit Instructions for Use (Handbook)*.
Suggested profile and training for users	
User profile	The *ipsogen* JAK2 RGQ PCR Kit is intended for professional use. The product is to be used only by professionals specially instructed, trained for molecular biology techniques and familiar with the device technology. The device procedure is to be implemented in a molecular biology laboratory environment.
User training	The product is to be used only by professionals specially instructed, trained for molecular biology techniques and familiar with the device technology. The device procedure is to be implemented in a molecular biology laboratory environment.
Risks and warnings	
Residual risks and undesirable effects	The relevant residual risks were identified and disclosed to the user in the form of warnings and precautions in the *ipsogen* JAK2 RGQ PCR Kit Instructions for Use: ● **Risk of contamination** Refer to "qPCR on Rotor-Gene Q MDx 5plex HRM instrument with 72-tube rotor" and "Precautions" Instructions for Use sections. ○ Use extreme caution to prevent DNA or PCR product carryover contamination resulting in a false-positive signal.

	○ Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents. ○ Be careful to change tips between each tube to avoid any non-specific template or reaction mix contamination and, therefore, false-positive results. Start by adding the test samples, then the standards and controls. ○ Use extreme caution to prevent contamination of the mixes with the synthetic materials that are contained in the JAK2 MT and JAK2 WT Quant Standards reagents and with the JAK2 Mutant and JAK2 WT Control reagents. ● **Risk of deterioration of kit's reagents leading to qPCR run failure** Refer to "qPCR on Rotor-Gene Q MDx 5plex HRM instrument with 72-tube rotor" > "Procedure" > "Setting up the qPCR experiment" Instructions for Use section. **Important**: Do not exceed 30 minutes for the thawing step to avoid any material degradation. ● **Risk of incorrect tube position into the rotor leading to aberrant results** Refer to "Sample processing on Rotor-Gene Q MDx 5plex HRM instrument with 72-tube rotor" Instructions for Use section. **Important**: Tubes must be inserted into the rotor as indicated in Figure 6 of *ipsogen JAK2 RGQ PCR Kit Instructions for Use* as the automated analysis set in the assay profile is based on this organization. If a different layout is used, aberrant results will be obtained.
Warnings and precautions	Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative

and the regulatory authority in which the user and/or the patient is established.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

- Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample or preparation waste.

Emergency information

- CHEMTREC
  Outside USA & Canada +1 703-527-3887

Precautions

- Use of qPCR tests requires good laboratory practices, including maintenance of equipment that are dedicated to molecular biology, and is compliant with applicable regulations and relevant standards.

	• This kit is intended for in vitro diagnostic use. Reagents and instructions supplied in this kit have been validated for optimal performance. • The test is for use with whole blood samples anti-coagulated with potassium EDTA (K_2-EDTA) and stored at 2–8°C for no more than 96 hours until DNA extraction. • All chemicals and biological materials are potentially hazardous. Specimens and samples are potentially infectious and must be treated as biohazardous materials. • Discard sample and assay waste according to your local safety procedures. • Reagents for *ipsogen* JAK2 RGQ PCR Kit are optimally diluted. Do not dilute reagents further as this may result in a loss of performance. • Do not use reaction volumes (reaction mix plus sample) of less than 25 µl. • All reagents supplied in the *ipsogen* JAK2 RGQ PCR Kit are intended to be used solely with the other reagents supplied in the same kit. Do not substitute any reagent from one kit with the same reagent from another *ipsogen* JAK2 RGQ PCR Kit, even from the same batch, as this may affect performance. • Refer to *Rotor-Gene Q MDx 5plex HRM User Manual*, *Rotor-Gene AssayManager v2.1 Core Application User Manual*, *Gamma Plug-In User Manual*, and QIAsymphony SP instrument user manual for additional warnings, precautions, and procedures. • Alteration of incubation times and temperatures may result in erroneous or discordant data. • Do not use expired or incorrectly stored components. • Reaction mixes may be altered if exposed to light. • Use extreme caution to prevent contamination of the mixes with the synthetic materials that are contained in the JAK2 MT and JAK2

	WT Quant Standards reagents and with the JAK2 Mutant and JAK2 WT Control reagents. ● Use extreme caution to prevent DNA or PCR product carryover contamination resulting in a false-positive signal. ● Use extreme caution to prevent contamination by DNase, which might cause degradation of the template DNA. ● Use individual, dedicated pipets for setting up reaction mixes and adding templates. ● Do not open the Rotor-Gene Q MDx instrument until the run is finished. ● Do not open the Rotor-Gene Q tubes after the run is finished. ● Caution must be observed to ensure correct sample testing with emphasis on wrong sample entry, loading error, and pipetting error. ● Make sure the samples are handled in a systematic way to ensure correct identification at all times to maintain traceability. We therefore recommend the following: ● Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay. ● Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents. ● Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrices (DNA, plasmid, or PCR products) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.). ● For troubleshooting and safety information relative to the extraction kits QIAamp DSP DNA Blood Mini Kit (cat. no. 61104) and

	QIAsymphony DNA DSP Mini Kit (cat. no. 937236), please refer to the corresponding Instructions for Use. ● For troubleshooting information relating to Rotor-Gene AssayManager v2.1, please refer to the *Rotor-Gene AssayManager v2.1 Core Application User Manual*. Additionally, refer to *ipsogen JAK2 RGQ PCR Kit Instructions for Use* sections for: ● "Reagent Storage and Handling" section: "Repeated thawing and freezing should be avoided. Do not exceed a maximum of five freeze–thaw cycles." ● "Automated genomic DNA extraction using the QIAsymphony DSP DNA Mini Kit" section: "If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing proteinase K with screw caps immediately after the end of the protocol run to avoid evaporation." ● "Limitations" ○ The kit is intended for professional use. ○ The product is to be used only by professionals specially instructed, trained for molecular biology techniques and familiar with the device technology. The device procedure is to be implemented in a molecular biology laboratory environment. ○ The *ipsogen* JAK2 RGQ PCR Kit is not an automated device; however, the analysis is assisted by a dedicated software for automatic mutation quantification. ○ This kit should be used following the instructions given in the *ipsogen JAK2 RGQ PCR Kit Instructions for Use*, in combination

	with a validated instrument mentioned in "Materials Required but Not Provided". ○ Attention should be paid to expiration dates printed on the box label and the tube labels. Do not use expired components. ○ All reagents supplied in the *ipsogen* JAK2 RGQ PCR Kit are intended to be used solely with the other reagents supplied in the same kit. Failing to follow this guideline might affect performance. ○ The *ipsogen* JAK2 RGQ PCR Kit is validated only for human peripheral whole blood anticoagulated with 2K-EDTA collected from patients with suspected or diagnosed MPN. ○ The *ipsogen* JAK2 RGQ PCR Kit is validated only for use with the QIAsymphony DNA DSP Mini Kit (cat. no. 937236) or the QIAamp DSP DNA Blood Mini Kit (cat. no. 61104). ○ The *ipsogen* JAK2 RGQ PCR Kit is validated only for use with the Rotor-Gene Q MDx 5plex HRM (for PCR) and the QIAsymphony SP (for sample preparation). ○ Any off-label use of this product and/or modification of the components will void QIAGEN's liability. ○ Any diagnostic results generated must be interpreted in conjunction with other clinical-pathological findings. The absence of the JAK2 V617F/G1849T mutation does not exclude the presence of other JAK2 mutations. The test can report false negative results in case of additional mutations located in nucleotides 88504 to 88622. ○ It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance studies ● "Performance characteristics, Interfering substances"

	○ The study design meets the requirements of the NCCLS standard EP7-A3 "Interference Testing in clinical Chemistry". A total of 19 substances potentially present in blood samples were chosen for their potential effect on PCR (busulfan, citalopram hydrobromide, paroxetine hydrochloride hemihydrate, sertraline hydrochloride, fluoxetine hydrochloride, acetaminophen [paracetamol], bilirubin unconjugated, potassium 2K EDTA and 3K EDTA, sodium EDTA, Hgb [human], triglycerides, lisinopril dehydrate, hydroxyurea, acetylsalicylic acid, salicylic acid, thiotepa, anagrelide, interferon alpha 2b). ○ Substances from the DNA extraction process were also assessed (QSL1, QSB1, QSW1, QSW2, and PK from the QIAsymphony DSP DNA Blood Mini Kit; QIAGEN Protease, ethanol, AW1 and AW2 from the QIAamp DSP DNA Blood Mini Kit). ○ The obtained results showed no interfering effect for these substances. ● "Troubleshooting Guide" The troubleshooting guide may be helpful in solving any problems that may arise for the following components of the workflow: ○ Sample processing and failure ○ Error in DNA concentration ○ Sample is invalid due to low concentration ○ *ipsogen* JAK2 RGQ PCR Kit user error ○ No or low signal in the sample and controls ○ Errors in the operation of the RGQ

	For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support** (for contact information, visit **www.qiagen.com**).