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# *ipsogen*<sup>®</sup> JAK2 MutaQuant<sup>®</sup> Kit Handbook

 12 (catalog no. 673522)

 24 (catalog no. 673523)

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene<sup>®</sup> Q, ABI PRISM<sup>®</sup> 7900HT SDS,  
Applied Biosystems<sup>®</sup> 7500 Real-Time PCR System, and  
LightCycler<sup>®</sup> instruments



REF

673522, 673523



QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

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## Intended Use

The *ipsogen* JAK2 MutaQuant Kit is an *in vitro* quantitative test intended for the detection and quantification of JAK2 V617F/G1849T allele in genomic DNA extracted from peripheral blood of subjects with suspected myeloproliferative neoplasm (MPN).

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 (1).

**Note:** The kit should be used following the instructions given in this manual, in combination with validated reagents and instruments. Any off-label use of this product and/or modification of the components will void QIAGEN's liability.

## Summary and Explanation

A recurrent somatic mutation, V617F, affecting the Janus tyrosine kinase 2 (JAK2) gene, has been identified in 2005 (2–5), leading to a major breakthrough in the understanding, classification, and diagnosis of myeloproliferative neoplasms (MPN). JAK2 is a critical intracellular signaling molecule for a number of cytokines, including erythropoietin.

The JAK2 V617F mutation is detected in >95% of patients with polycythemia vera (PV), 50–60% of patients with essential thrombocythemia (ET), and in 50% of patients with primary myelofibrosis (PMF). JAK2 V617F has been also detected in some rare cases of chronic myelomonocytic leukemia, myelodysplastic syndrome, systemic mastocytosis, and chronic neutrophilic leukemia, but in 0% of CML (6).

The mutation corresponds to a single-nucleotide change of JAK2 nucleotide 1849 in exon 14, resulting in a unique valine (V) to phenylalanine (F) substitution at position 617 of the protein (JH2 domain). It leads to constitutive activation of JAK2, hematopoietic transformation *in vitro*, and erythropoietin-independent erythroid colony (EEC) growth in all patients with PV and a large proportion of ET and PMF patients (7). JAK2 V617F represents a key driver in the transformation of hematopoietic cells in MPN, but the exact pathological mechanisms leading, with the same unique mutation, to such different clinical and biological entities remain to be fully elucidated.

Traditionally, the diagnosis of MPNs was based on clinical, bone marrow histology and cytogenetic criteria. The discovery of a disease-specific molecular marker resulted in both simplification of the process and increased diagnostic accuracy. Detection of the JAK2 V617F mutation is now part of the reference WHO 2008 criteria for the diagnosis of BCR-ABL negative MPN (Table 1), and presence of this mutation is a major criterion for diagnostic confirmation.

**Table 1. WHO criteria for the diagnosis of MPN (adapted from reference 8)**

|  |  |
|--|--|
| Criteria for a diagnosis of polycythemia vera (PV)         |  |
| Major  | <p>1. Hemoglobin (Hgb) &gt;18.5 g.dl<sup>-1</sup> (men) or &gt;16.5 g.dl<sup>-1</sup> (women) or Hgb or hematocrit (Hct) &gt;99th percentile of reference range for age, sex, or altitude of residence or Hgb &gt;17 g.dl<sup>-1</sup> (men) or &gt;15 g.dl<sup>-1</sup> (women) if associated with sustained increase of ≥2 g.dl<sup>-1</sup> from baseline that cannot be attributed to correction of iron deficiency or Elevated red cell mass &gt;25% above mean normal predicted value</p> <p>2. Presence of <i>JAK2V617F</i> or similar mutation</p> |
| Minor  | <p>1. Bone marrow trilineage myeloproliferation</p> <p>2. Subnormal serum erythropoietin level</p> <p>3. Endogenous erythroid colony (EEC) growth</p>  |
| Criteria for a diagnosis of essential thrombocythemia (ET) |  |
| Major  | <p>1. Platelet count ≥450 x 10<sup>9</sup> l<sup>-1</sup></p> <p>2. Megakaryocyte proliferation with large and mature morphology. No or little granulocyte or erythroid proliferation</p> <p>3. Not meeting WHO criteria for chronic myeloid leukemia (CML), PV, primary myelofibrosis (PMF), myelodysplastic syndrome (MDS), or other myeloid neoplasm</p> <p>4. Demonstration of <i>JAK2V617F</i> or other clonal marker or No evidence of reactive thrombocytosis</p>   |
| Minor  | -  |
| Criteria for a diagnosis of primary myelofibrosis (PMF)    |  |
| Major  | <p>1. Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis or In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (i.e. prefibrotic PMF)</p> <p>2. Not meeting WHO criteria for (CML), PV, MDS, or other myeloid neoplasm</p> <p>3. Demonstration of <i>JAK2V617F</i> or other clonal marker or No evidence of reactive marrow fibrosis</p>                                      |
| Minor  | <p>1. Leukoerythroblastosis</p> <p>2. Increased serum lactate dehydrogenase (LDH)</p> <p>3. Anemia</p> <p>4. Palpable splenomegaly</p>   |

Recently, international experts have proposed criteria for therapeutic trials in PV and ET. Based on data on allograft, alpha-interferon, or hydroxyurea, *JAK2V617F* quantification has been incorporated as a potentially useful tool to monitor treatment response (9). A decrease in *JAK2 V617F* burden has been

observed in response to some of the new anti-JAK2 targeted drugs in clinical development (10).

## Principle of the Procedure

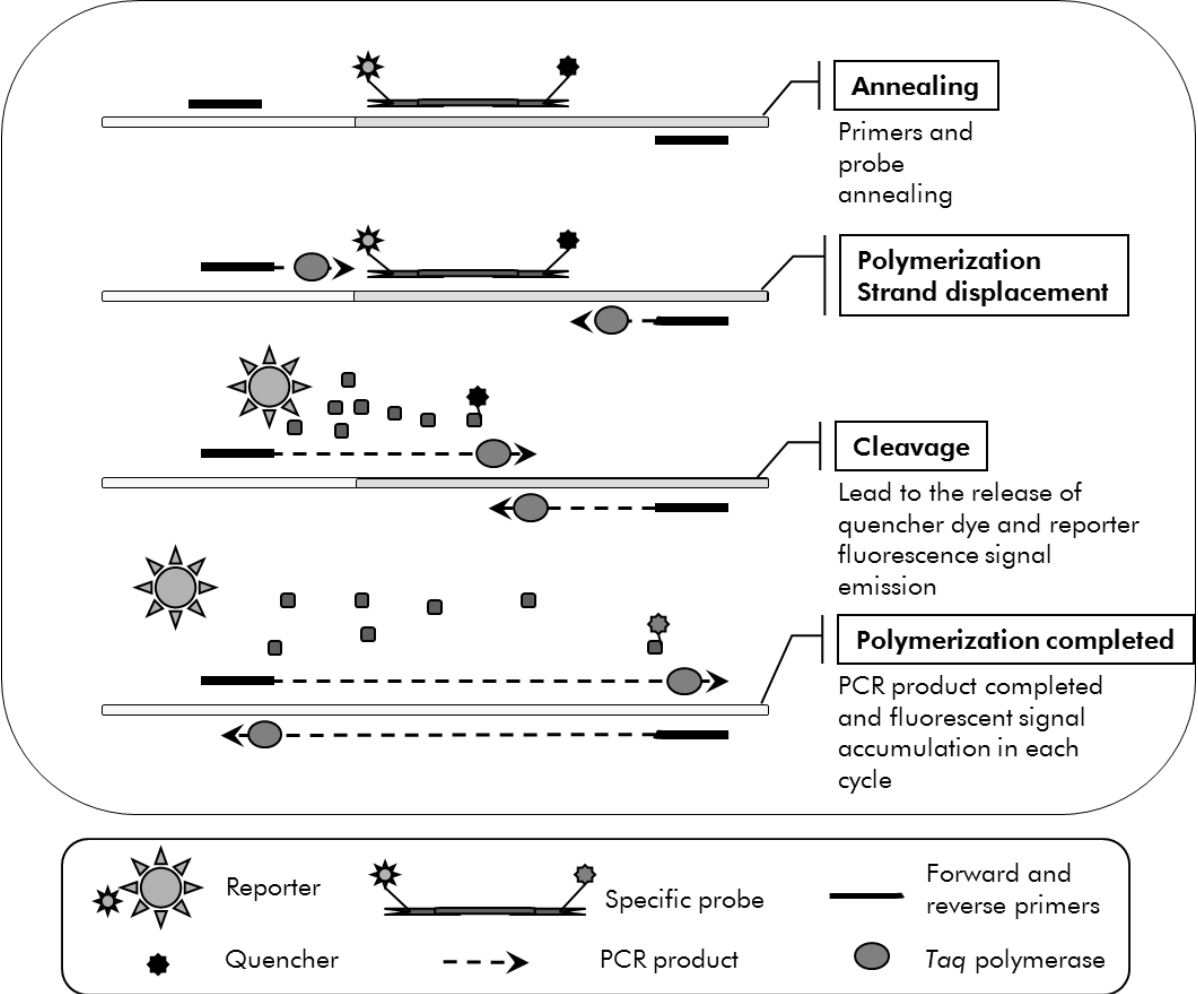
Several different techniques have been proposed to quantitatively determine the proportion of single nucleotide polymorphisms (SNPs) in DNA samples. Of these, methods based on real-time quantitative polymerase chain reaction (qPCR) are preferred because of their higher sensitivity allowing monitoring of allele burden in a longitudinal fashion. Many of these techniques have a moderate sensitivity of 1–10%, for example, TaqMan<sup>®</sup> allelic discrimination, Pyrosequencing<sup>®</sup>, melting curve assay, and direct sequencing. Some, such as melting curve and sequencing, are only semiquantitative while others, such as Pyrosequencing, require post-PCR processing or need instrumentation that is not readily available or has prohibitively high setup costs for routine laboratory testing. A highly sensitive approach with a sensitivity <0.1% requires the use of a SNP specific primer that permits the selective amplification of the mutant or wild type allele that is easily detectable on a real-time qPCR instrument. The *ipsogen* JAK2 MutaQuant Kit is based on this technique.

The use of qPCR permits the accurate quantification of PCR products during the exponential phase of the PCR amplification process. Quantitative PCR data can be rapidly obtained, without post-PCR processing, by real-time detection of fluorescent signals during and/or subsequent to PCR cycling, thereby drastically reducing the risk of PCR product contamination. At present, 3 main types of qPCR techniques are available: qPCR analysis using SYBR<sup>®</sup> Green I Dye, qPCR analysis using hydrolysis probes, and qPCR analysis using hybridization probes.

This assay exploits the qPCR double-dye oligonucleotide hydrolysis principle. During PCR, forward and reverse primers hybridize to a specific sequence. A double-dye oligonucleotide is contained in the same mix. This probe, which consists of an oligonucleotide labeled with a 5' reporter dye and a downstream, 3'quencher dye, 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 dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer.

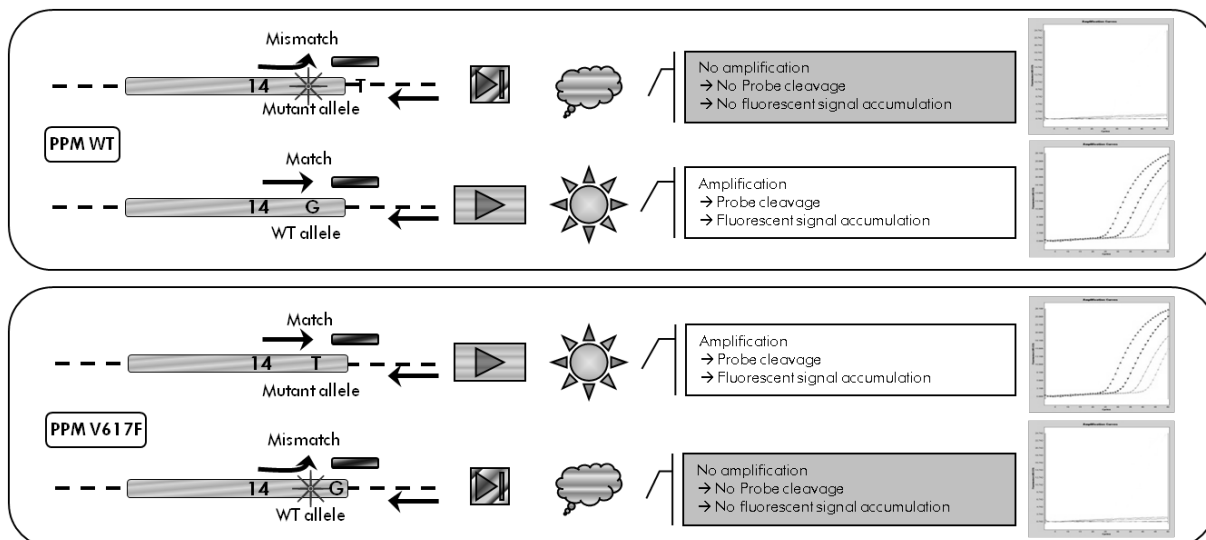
During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'→3' exonuclease activity of the DNA polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR (Figure 1). This process occurs in every cycle and does not interfere with the exponential accumulation of product.

The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and hence amplified during PCR. Because of these requirements, nonspecific amplification is not detected. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.



**Figure 1. Reaction principle.**

The quantitative allele specific PCR technology used in this assay kit allows a sensitive, accurate, and highly reproducible detection of SNPs. This technique is based on the use of specific forward primers, for the wild-type and the V617F allele (11). Only perfect match between primer and target DNA allows extension and amplification in the PCR (see Figure 2).



**Figure 2. Allele specific PCR.** Use of wild-type or the V617F primers and probe mix allows the specific detection of the wild-type or mutated allele in two separate reactions conducted using the same sample. Results can be expressed as percentage of VF copies among total JAK2 copies.



# Materials Provided

## Kit contents

| <b>ipsogen JAK2 MutaQuant Kit</b>   |  | <b>(12)</b>   | <b>(24)</b>   |
|---|--|---------------|---------------|
| <b>Catalog no.</b>  |  | <b>673522</b> | <b>673523</b> |
| <b>Number of reactions</b>  |  | <b>12</b>     | <b>24</b>     |
| V617F positive control<br>(100% V617F allele)   | PC-VF-JAK2<br>PC-VF-JAK2 Mini                    | 40 $\mu$ l    | 60 $\mu$ l    |
| V617F negative control<br>(100% wild-type allele)   | NC-VF-JAK2<br>NC-VF-JAK2 Mini                    | 40 $\mu$ l    | 60 $\mu$ l    |
| M1-VF Standard Dilution, 50 copies<br>(5 x 10 <sup>1</sup> V617F copies/5 $\mu$ l)        | M1-VF<br>M1-VF Mini                              | 20 $\mu$ l    | 30 $\mu$ l    |
| M2-VF Standard Dilution, 500<br>copies (5 x 10 <sup>2</sup> V617F copies/5 $\mu$ l)       | M2-VF<br>M2-VF Mini                              | 20 $\mu$ l    | 30 $\mu$ l    |
| M3-VF Standard Dilution, 5000<br>copies (5 x 10 <sup>3</sup> V617F copies/5 $\mu$ l)      | M3-VF<br>M3-VF Mini                              | 20 $\mu$ l    | 30 $\mu$ l    |
| M4-VF Standard Dilution, 50,000<br>copies (5 x 10 <sup>4</sup> V617F copies/5 $\mu$ l)    | M4-VF<br>M4-VF Mini                              | 20 $\mu$ l    | 30 $\mu$ l    |
| WT-1 Standard Dilution, 50 copies<br>(5 x 10 <sup>1</sup> wild-type copies/5 $\mu$ l)     | WT-1<br>WT-1 Mini                                | 20 $\mu$ l    | 30 $\mu$ l    |
| WT-2 Standard Dilution, 500 copies<br>(5 x 10 <sup>2</sup> wild-type copies/5 $\mu$ l)    | WT-2<br>WT-2 Mini                                | 20 $\mu$ l    | 30 $\mu$ l    |
| WT-3 Standard Dilution, 5000<br>copies (5 x 10 <sup>3</sup> wild-type copies/5 $\mu$ l)   | WT-3<br>WT-3 Mini                                | 20 $\mu$ l    | 30 $\mu$ l    |
| WT-4 Standard Dilution, 50,000<br>copies (5 x 10 <sup>4</sup> wild-type copies/5 $\mu$ l) | WT-4<br>WT-4 Mini                                | 20 $\mu$ l    | 30 $\mu$ l    |
| Primers and Probe Mix JAK2 WT*  | PPM-JAK2 WT 25x<br>PPM-JAK2 WT Mini 25x          | 52 $\mu$ l    | 95 $\mu$ l    |
| Primers and Probe Mix JAK2 V617F <sup>†</sup>   | PPM-JAK2 V617F 25x<br>PPM-JAK2 V617F Mini<br>25x | 52 $\mu$ l    | 95 $\mu$ l    |
| ipsogen JAK2 MutaQuant Kit Handbook (English)   |  | 1             | 1             |

\* Mix of specific reverse and forward primers for the wild-type JAK2 control gene plus a specific FAM™–TAMRA™ probe.

<sup>†</sup> Mix of specific reverse and forward primers for the JAK2 V617F mutation plus a specific FAM–TAMRA probe.

**Note:** Vortex and briefly centrifuge the standard dilutions and the primers and probe mixes before use.

## Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

### Reagents

- Nuclease-free PCR grade water
- Buffer and *Taq* DNA polymerase: The validated reagents are TaqMan Universal PCR Master Mix (Master Mix PCR 2x) (Life Technologies, cat. no. 4304437) and LightCycler TaqMan Master (Master Mix PCR 5x) (Roche, cat. no. 04535286001) or LightCycler FastStart DNA Master<sup>PLUS</sup> HybProbe<sup>®</sup> (Master Mix 5x) (Roche, cat. No. 03515567001)

### Consumables

- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 1.5 ml nuclease-free PCR tubes
- Ice

### Equipment

- Microliter pipet\* dedicated for PCR (1–10  $\mu$ l; 10–100  $\mu$ l; 100–1000  $\mu$ l)
- Benchtop centrifuge\* with rotor for 0.5 ml/1.5 ml reaction tubes and microplates (capable of attaining 13,000–14,000 rpm)
- Real-time PCR instrument:\* Rotor-Gene Q 5plex HRM or other Rotor-Gene; LightCycler 1.2, or 480; ABI PRISM 7900HT SDS; Applied Biosystems 7500 Real-Time PCR System; and associated specific material
- Biophotometer

\* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

## Warnings and Precautions

For in vitro diagnostic use

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](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Discard sample and assay waste according to your local safety regulations.

### General precautions

Use of qPCR tests require 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. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. PPM-WT and PPM-VF reagents may be altered if exposed to light. All reagents are formulated specifically for use with this test. For optimal performance of the test, no substitutions should be made.

Use extreme caution to prevent:

- DNase contamination that might cause degradation of the template DNA
- DNA or PCR carryover contamination resulting in false positive signal

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 matrixes (DNA, plasmid, or PCR products) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).

## Reagent Storage and Handling

The kits are shipped on dry ice and must be stored at  $-30^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPM-WT and PPM-VF tubes).

- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

There are no obvious signs to indicate instability of this product. However, positive and negative controls should be run simultaneously with unknown specimens.

## Procedure

### Sample DNA preparation

Genomic DNA should be obtained either from whole blood, purified peripheral blood lymphocytes of whole blood, polynuclear cells, or granulocytes. For comparable results it is recommended that the same cellular fraction and DNA extraction method are used. DNA extraction can be performed using a home-brew method or a commercially available kit.

DNA quantity should be determined by measuring the optical density (OD) of the sample at 260 nm and DNA quality can be determined either by spectrophotometry or gel\* electrophoresis.

- The  $OD_{260}/OD_{280}$  ratio should be 1.7–1.9 and smaller ratios than this may indicate protein contamination or the presence of organic chemicals.
- Electrophoretic analysis on a 0.8–1.0% agarose gel\* should allow the visualization of the isolated DNA as a distinct band of approximately 20 kb (a slight smear will give acceptable results).

The resultant DNA will need to be diluted to a concentration of 5 ng/ $\mu$ l in 1x TE buffer\* at pH 8.0 and then stored at +4 to +8°C for 1 week or at –20°C if longer term storage is required.

The qPCR reaction is optimized for DNA samples containing 25 ng purified genomic DNA.

\* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

## Protocol: qPCR on Rotor-Gene Q MDx 5plex HRM or Rotor-Gene Q 5plex HRM instruments with 72-tube rotor

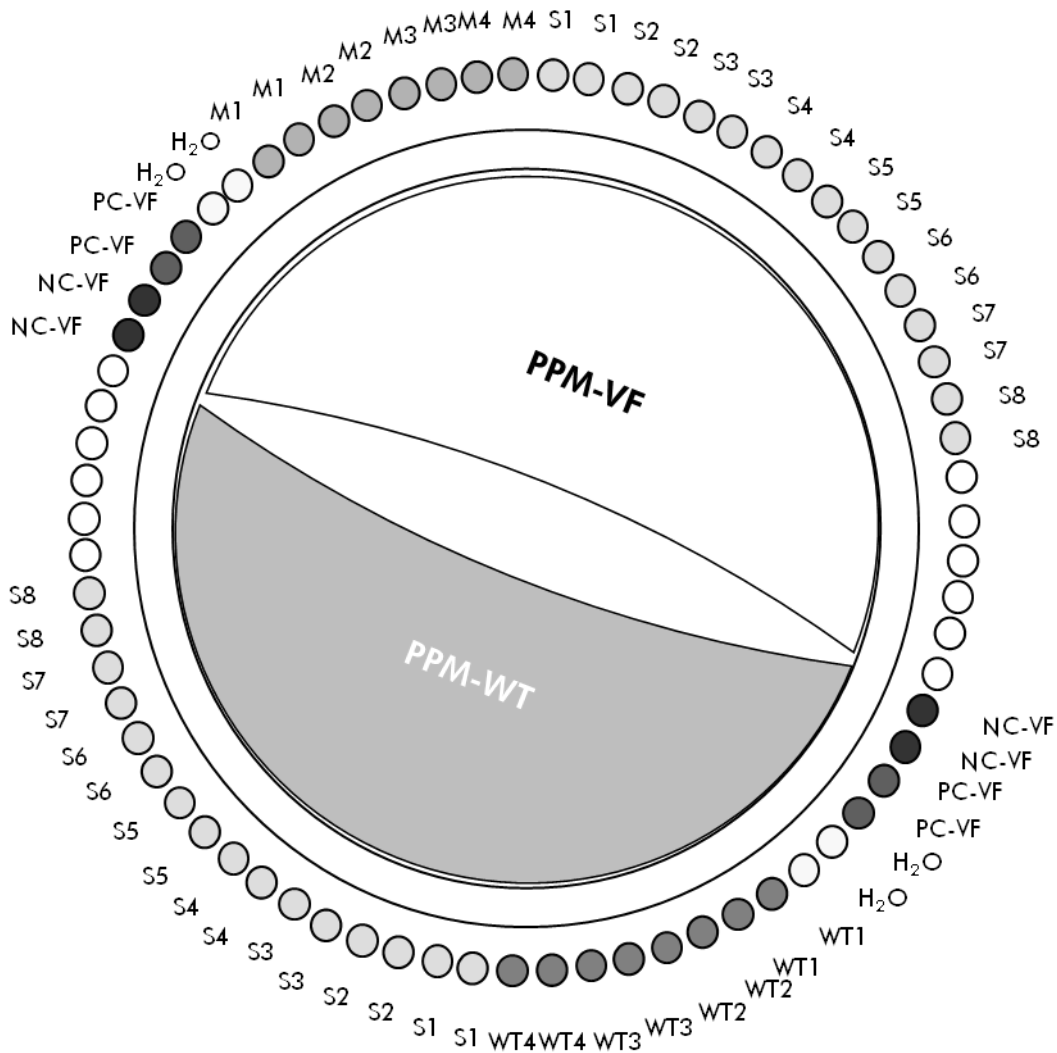
Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 2.

**Table 2. Number of reactions for Rotor-Gene Q instruments with 72-tube rotor**

| <b>Samples</b>  | <b>Reactions</b>   |
|---|--|
| <b>With the JAK2 V617F primers and probe mix (PPM-VF)</b>     |  |
| 4 M-VF standards  | 8 reactions, each one tested in duplicate  |
| n DNA samples   | n x 2 reactions  |
| 2 DNA controls  | 4 reactions: positive control (PC-VF) and negative control (NC-VF), each one tested in duplicate |
| Water control   | 2 reactions  |
| <b>With the JAK2 wild-type primers and probe mix (PPM-WT)</b> |  |
| 4 wild-type standards   | 8 reactions, each one tested in duplicate  |
| n DNA samples   | n x 2 reactions  |
| 2 DNA controls  | 4 reactions: PC-VF and NC-VF, each one tested in duplicate                                       |
| Water control   | 2 reactions  |

### Sample processing on Rotor-Gene Q instruments with 72-tube rotor

We recommend testing at least 8 DNA samples with the 24 reaction kit (catalog no. 673523) and at least 6 DNA samples with the 12 reaction kit (catalog no. 673522) in the same experiment to optimize the use of the standards and the primers and probe mixes.



**Figure 3. Suggested rotor setup for each experiment with the *ipsogen 24* sample JAK2 MutaQuant Kit.** PC-VF: V617F positive control; NC-VF: V617F negative control; M-VF: V617F standards; M-WT: wild-type standards; S: DNA sample; H<sub>2</sub>O: water control.

**Note:** Take care to always place a sample to be tested in position 1 of the rotor. Otherwise, during the calibration step, the instrument will not perform calibration, and incorrect fluorescence data will be acquired.

Fill all other positions with empty tubes.

### qPCR on Rotor-Gene Q instruments with 72-tube rotor

**Note:** Perform all steps on ice.

#### Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following qPCR mix according to the number of samples being processed.

All concentrations are for the final volume of the reaction.

Table 3 and 4 describe the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25  $\mu$ l. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT). Extra volumes are included to compensate for pipetting error.

**Table 3. Preparation of qPCR mix**

| <b>Component</b>                    | <b>1 reaction<br/>(<math>\mu</math>l)</b> | <b>V617F pre-mix<br/>30 + 1<br/>reactions (<math>\mu</math>l)</b> | <b>Final<br/>concentration</b> |
|-------------------------------------|---|---|--------------------------------|
| TaqMan Universal PCR Master Mix, 2x | 12.5                                      | 387.5   | 1x                             |
| Primers and probe mix, PPM-VF 25x   | 1.0                                       | 31  | 1x                             |
| Nuclease-free PCR grade water       | 6.5                                       | 201.5   | –                              |
| Sample (to be added at step 4)      | 5.0                                       | 5 each  | –                              |
| Total volume                        | 25.0                                      | 25 each   | –                              |



**Table 4. Preparation of qPCR mix**

| <b>Component</b>                    | <b>1 reaction<br/>(<math>\mu</math>l)</b> | <b>WT pre-mix<br/>30 + 1<br/>reactions (<math>\mu</math>l)</b> | <b>Final<br/>concentration</b> |
|-------------------------------------|---|--|--------------------------------|
| TaqMan Universal PCR Master Mix, 2x | 12.5                                      | 387.5  | 1x                             |
| Primers and probe mix, PPM-WT 25x   | 1.0                                       | 31   | 1x                             |
| Nuclease-free PCR grade water       | 6.5                                       | 201.5  | –                              |
| Sample (to be added at step 4)      | 5.0                                       | 5 each   | –                              |
| Total volume                        | 25.0                                      | 25 each  | –                              |

3. Dispense 20  $\mu$ l of the qPCR pre-mix (VF or WT) per tube.
4. Add 5  $\mu$ l of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding tube (total volume 25  $\mu$ l).
5. Mix gently, by pipetting up and down.
6. Place the tubes in the thermal cycler according to the manufacturer recommendations.
7. Program the Rotor-Gene Q instrument with the thermal cycling program as indicated in Table 5.

**Table 5. Temperature profile**

|                         |  |
|-------------------------|--|
| <b>Mode of analysis</b> | Quantitation   |
| <b>Hold</b>             | Temperature: 50 deg<br>Time: 2 mins  |
| <b>Hold 2</b>           | Temperature: 95°C<br>Time: 10 mins   |
| <b>Cycling</b>          | 50 times<br>95°C for 15 secs<br>62°C for 1 min with acquisition of FAM fluorescence in channel Green: Single |

- 8. For Rotor-Gene Q instruments, select “Slope Correct” for the analysis. We recommend setting the threshold at 0.03. Start the thermal cycling program, as indicated in Table 5.**

## Protocol: qPCR on ABI PRISM 7900HT SDS, Applied Biosystems 7500 Real-Time PCR System, and LightCycler 480 instrument

Using 96-well-plate qPCR equipment, we recommend performing all measurements in duplicate, as indicated in Table 6.

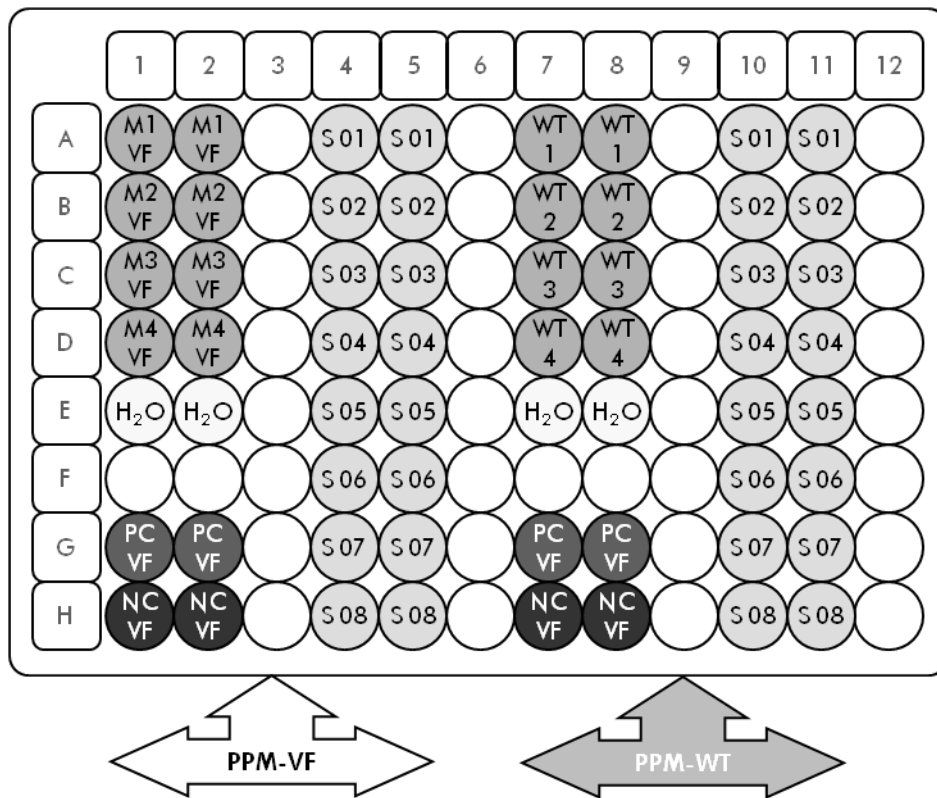
**Table 6. Number of reactions using 96-well-plate qPCR equipment**

| <b>Samples</b>  | <b>Reactions</b>   |
|---|--|
| <b>With the JAK2 V617F primers and probe mix (PPM-VF)</b>     |  |
| 4 M-VF standards  | 8 reactions, each one tested in duplicate                  |
| n DNA samples   | n x 2 reactions  |
| 2 DNA controls  | 4 reactions: PC-VF and NC-VF, each one tested in duplicate |
| Water control   | 2 reactions  |
| <b>With the JAK2 wild-type primers and probe mix (PPM-WT)</b> |  |
| 4 wild-type standards   | 8 reactions, each one tested in duplicate                  |
| n DNA samples   | n x 2 reactions  |
| 2 DNA controls  | 4 reactions: PC-VF and NC-VF, each one tested in duplicate |
| Water control   | 2 reactions  |

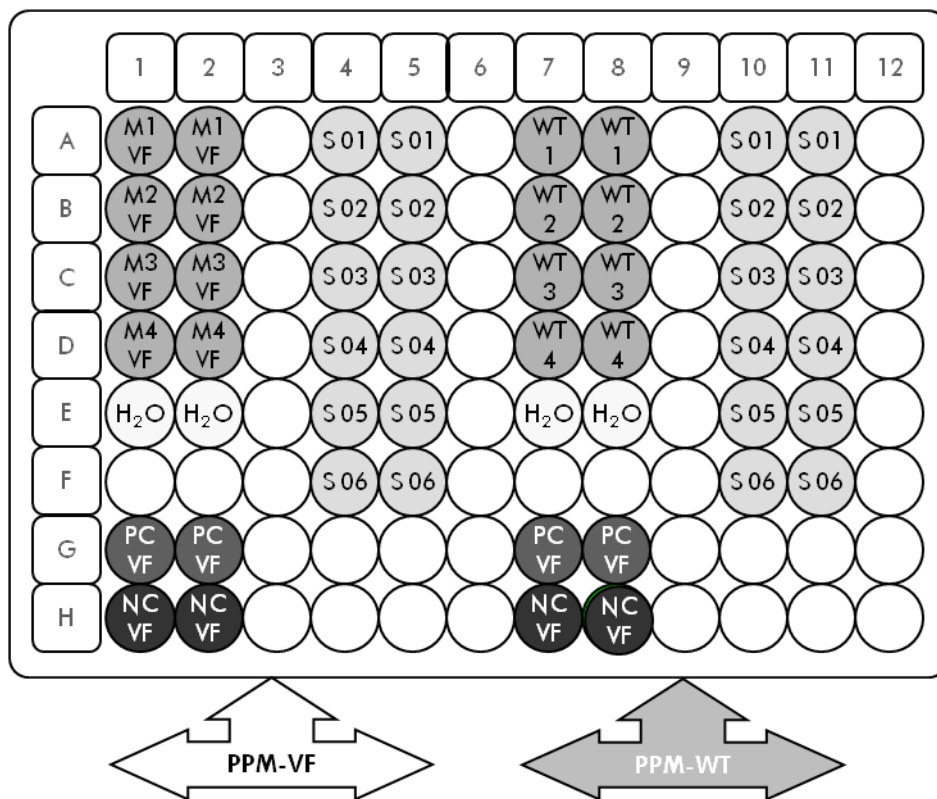
### Sample processing on ABI PRISM 7900HT SDS, Applied Biosystems 7500 Real-Time PCR System, and LightCycler 480 instrument

We recommend testing 8 DNA samples with the 24 reaction kit (catalog no. 673523) and at least 6 DNA samples with the 12 reaction kit (catalog no. 673522) in the same experiment to optimize the use of the standards and the primers and probe mixes.

The plate scheme in Figure 4 shows an example of such an experiment using the 24 reaction kit (catalog no. 673523), and Figure 5 shows an example of such an experiment using the 12 reaction kit (catalog no. 673522).



**Figure 4. Suggested plate setup for one experiment using the 24 reaction kit (catalog no. 673523).** PC-VF: V617F positive control; NC-VF: V617F negative control; M-VF: V617F standards; M-WT: wild-type standards; S: DNA sample; H<sub>2</sub>O: water control



**Figure 5. Suggested plate setup for one experiment using the 12 reaction kit (catalog no. 673522).** PC-VF: V617F positive control; NC-VF: V617F negative control; M-VF: V617F standards; M-WT: wild-type standards; S: DNA sample; H<sub>2</sub>O: water control

## qPCR on ABI PRISM 7900HT SDS, Applied Biosystems 7500 Real-Time PCR System, and LightCycler 480 instrument

**Note:** Perform all steps on ice.

### Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following qPCR mix according to the number of samples being processed.

All concentrations are for the final volume of the reaction.

Table 7 and 8 describe the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25  $\mu$ l. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT). Extra volumes are included to compensate for pipetting error.

**Table 7. Preparation of qPCR mix**

| Component                           | V617F pre-mix         |                             |                             | Final concentration |
|-------------------------------------|-----------------------|-----------------------------|-----------------------------|---------------------|
|                                     | 1 reaction ( $\mu$ l) | 26 + 1 reactions ( $\mu$ l) | 30 + 1 reactions ( $\mu$ l) |                     |
| TaqMan Universal PCR Master Mix, 2x | 12.5                  | 337.5                       | 387.5                       | 1x                  |
| Primers and probe mix, PPM-VF 25x   | 1.0                   | 27                          | 31                          | 1x                  |
| Nuclease-free PCR grade water       | 6.5                   | 175.5                       | 201.5                       | –                   |
| Sample (to be added at step 4)      | 5.0                   | 5 each                      | 5 each                      | –                   |
| Total volume                        | 25.0                  | 25 each                     | 25 each                     | –                   |

**Table 8. Preparation of qPCR mix**

| <b>Component</b>                    | <b>WT pre-mix</b>                     |   |   | <b>Final concentration</b> |
|-------------------------------------|---------------------------------------|---|---|----------------------------|
|                                     | <b>1 reaction (<math>\mu</math>l)</b> | <b>26 + 1 reactions (<math>\mu</math>l)</b> | <b>30 + 1 reactions (<math>\mu</math>l)</b> |                            |
| TaqMan Universal PCR Master Mix, 2x | 12.5                                  | 337.5                                       | 387.5                                       | 1x                         |
| Primers and probe mix, PPM-WT 25x   | 1.0                                   | 27  | 31  | 1x                         |
| Nuclease-free PCR grade water       | 6.5                                   | 175.5                                       | 201.5                                       | –                          |
| Sample (to be added at step 4)      | 5.0                                   | 5 each                                      | 5 each                                      | –                          |
| Total volume                        | 25.0                                  | 25 each                                     | 25 each                                     | –                          |

- 3. Dispense 20  $\mu$ l of the qPCR pre-mix (VF or WT) per well.**
- 4. Add 5  $\mu$ l of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding well (total volume 25  $\mu$ l).**
- 5. Mix gently, by pipetting up and down.**
- 6. Close the plate and briefly centrifuge (300 x g, approximately 10 seconds).**
- 7. Place the plate in the thermal cycler according to the manufacturer recommendations.**
- 8. Program the thermal cycler with the thermal cycling program and set the instrument for the acquisition of dual labeled FAM fluorescent probe as indicated in Table 9 for ABI PRISM 7900HT SDS and Applied Biosystems 7500 Real-Time PCR System, or Table 10 for the LightCycler 480 instrument.**

**Table 9. Temperature profile for ABI PRISM 7900HT SDS and Applied Biosystems 7500 Real-Time PCR System**

|                         |   |
|-------------------------|---|
| <b>Mode of analysis</b> | Standard Curve — Absolute Quantitation  |
| <b>Hold</b>             | Temperature: 50°C<br>Time: 2 minutes  |
| <b>Hold 2</b>           | Temperature: 95°C<br>Time: 10 minutes   |
| <b>Cycling</b>          | 50 times<br>95°C for 15 seconds<br>63°C for 1 minute 30 seconds with acquisition of FAM fluorescence; quencher: TAMRA |

**Table 10. Temperature profile for LightCycler 480 instrument**

|                          |   |
|--------------------------|---|
| <b>Mode of analysis</b>  | Absolute Quantification (“Abs Quant”)   |
| <b>Detection formats</b> | Select “Simple Probe” in the Detection formats window   |
| <b>Hold</b>              | Temperature: 50°C<br>Time: 2 minutes  |
| <b>Hold 2</b>            | Temperature: 95°C<br>Time: 10 minutes   |
| <b>Cycling</b>           | 50 times<br>95°C for 15 seconds<br>63°C for 1 minute 30 seconds with acquisition of FAM fluorescence corresponding to (483–533 nm) for LC version 01 and (465–510 nm) for LC version 02 |

**9. For the ABI PRISM 7900HT and Applied Biosystems 7500 Real-Time PCR System, follow step 8a. For the LightCycler 480 instrument, follow step 8b.**

**9a. ABI PRISM 7900HT and Applied Biosystems 7500 Real-Time PCR System: We recommend a threshold set at 0.1. Start the cycling program, as indicated in Table 9.**

**9b. LightCycler 480: We recommend a Fit point analysis mode with background at 2.0 and threshold at 2.0. Start the thermal cycling program, as indicated in Table 10.**



## Protocol: qPCR on LightCycler 1.2 instrument

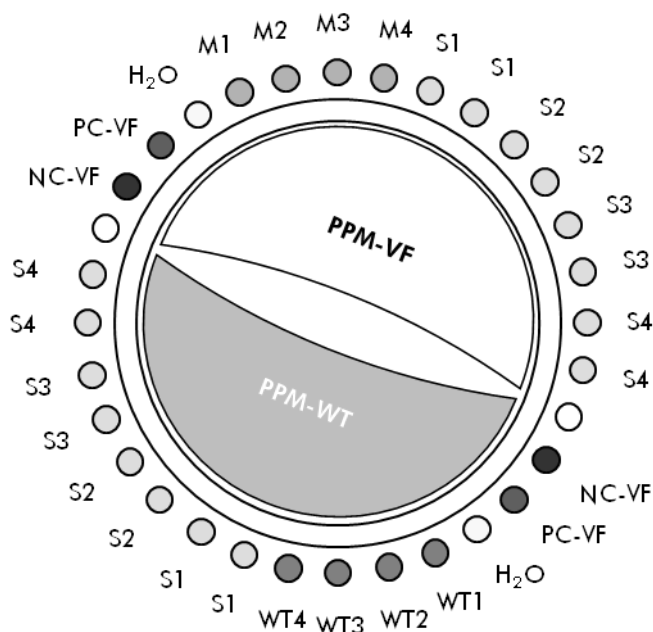
Using capillary instruments, we recommend measuring samples in duplicate and controls only once, as indicated in Table 11.

**Table 11. Number of reactions for LightCycler 1.2 instrument**

| <b>Samples</b>  | <b>Reactions</b>                                   |
|---|--|
| <b>With the JAK2 V617F primers and probe mix (PPM-VF)</b>     |  |
| 4 M-VF standards  | 4 reactions, each tested once                      |
| n DNA samples   | n x 2 reactions                                    |
| 2 DNA controls  | 2 reactions: PC-VF and NC-VF, each tested once     |
| Water control   | 1 reaction   |
| <b>With the JAK2 wild-type primers and probe mix (PPM-WT)</b> |  |
| 4 wild-type standards   | 4 reactions, each one tested once                  |
| n DNA samples   | n x 2 reactions                                    |
| 2 DNA controls  | 2 reactions: PC-VF and NC-VF, each one tested once |
| Water control   | 1 reaction   |

### Sample processing on LightCycler 1.2 instrument

We recommend testing 4 DNA samples in the same experiment to optimize the use of the standards and primers and probe mixes. The capillary scheme in Figure 6 shows an example of an experiment.



**Figure 6. Suggested rotor setup for each experiment with the *ipsogen* JAK2 MutaQuant Kit.** PC-VF: V617F positive control; NC-VF: V617F negative control; M-VF: V617F standards; M-WT: wild-type standards; S: DNA sample; H<sub>2</sub>O: water control.

## qPCR on LightCycler 1.2 instrument

**Note:** Because of particular technological requirements, LightCycler experiments must be performed using specific reagents. We recommend the use of LightCycler FastStart DNA Master<sup>PLUS</sup> HybProbe and following the manufacturer's instructions to prepare the Master Mix 5x.

**Note:** Perform all steps on ice.

## Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following qPCR mix according to the number of samples being processed.

All concentrations are for the final volume of the reaction.

Table 12 and 13 describe the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 20  $\mu$ l. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix (either PPM-VF or PPM-WT). Extra volumes are included to compensate for pipetting error.

**Table 12. Preparation of qPCR mix**

| <b>Component</b>  | <b>1 reaction<br/>(<math>\mu</math>l)</b> | <b>V617F pre-mix<br/>15 + 1<br/>reactions (<math>\mu</math>l)</b> | <b>Final<br/>concentration</b> |
|---|---|---|--------------------------------|
| Freshly prepared<br>LightCycler FastStart<br>DNA Master <sup>PLUS</sup><br>HybProbe Mix, 5x | 4.0                                       | 64.0  | 1x                             |
| Primers and probe<br>mix, PPM-VF<br>25x   | 0.8                                       | 12.8  | 1x                             |
| Nuclease-free PCR<br>grade water  | 10.2                                      | 163.2   | –                              |
| Sample (to be<br>added at step 4)   | 5.0                                       | 5 each  | –                              |
| Total volume  | 20.0                                      | 20 each   | –                              |

**Table 13. Preparation of qPCR mix**

| <b>Component</b>  | <b>1 reaction<br/>(<math>\mu</math>l)</b> | <b>WT pre-mix<br/>15 + 1<br/>reactions (<math>\mu</math>l)</b> | <b>Final<br/>concentration</b> |
|---|---|--|--------------------------------|
| Freshly prepared<br>LightCycler FastStart<br>DNA Master <sup>PLUS</sup><br>HybProbe Mix, 5x | 4.0                                       | 64.0   | 1x                             |
| Primers and probe<br>mix, PPM-WT<br>25x   | 0.8                                       | 12.8   | 1x                             |
| Nuclease-free PCR<br>grade water  | 10.2                                      | 163.2  | –                              |
| Sample (to be<br>added at step 4)   | 5.0                                       | 5 each   | –                              |
| Total volume  | 20.0                                      | 20 each  | –                              |

3. Dispense 15  $\mu$ l of the qPCR pre-mix (VF or WT) per capillary.
4. Add 5  $\mu$ l of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding tube (total volume 20  $\mu$ l).
5. Mix gently, by pipetting up and down.
6. Place the capillaries in the adapters provided with the apparatus, and briefly centrifuge (700 x g, approximately 10 seconds).
7. Load the capillaries into the thermal cycler according to the manufacturer recommendations.
8. Program the LightCycler 1.2 instrument with the thermal cycling program as indicated in Table 14.

**Table 14. Temperature profile**

| <b>Mode of analysis</b> | Quantification   |
|-------------------------|--|
| <b>Hold 1</b>           | Temperature: 55°C<br>Time: 2 minutes<br>Ramp: 20   |
| <b>Hold 2</b>           | Temperature: 95°C<br>Time: 10 minutes<br>Ramp: 20  |
| <b>Cycling</b>          | 50 times<br>95°C for 15 seconds; ramp: 20<br>66°C for 1 minute; ramp: 20; with acquisition of FAM fluorescence: Single |

9. For the LightCycler 1.2, the F1/F2 and “2<sup>nd</sup> derivative analysis” mode is recommended. Start the thermal cycling program, as indicated in Table 14.

# Interpretation of Results

## Data analysis principle

Data for the threshold cycle ( $C_T$ ) and crossing point ( $C_p$ ) values can be exported from the qPCR instrument and pasted into an Excel<sup>®</sup> file for analysis. These values can then be used to calculate the mean value for  $C_p$  and  $C_T$  and the standard mean  $C_T$  values can be plotted to obtain a standard curve for both the wild-type and V617F standards using the following equation and Table 15.

$y = \text{Mean } C_p$ ;  $x = \log_{10} \text{CN}$  where CN = gene copy number in the 5  $\mu\text{l}$  sample

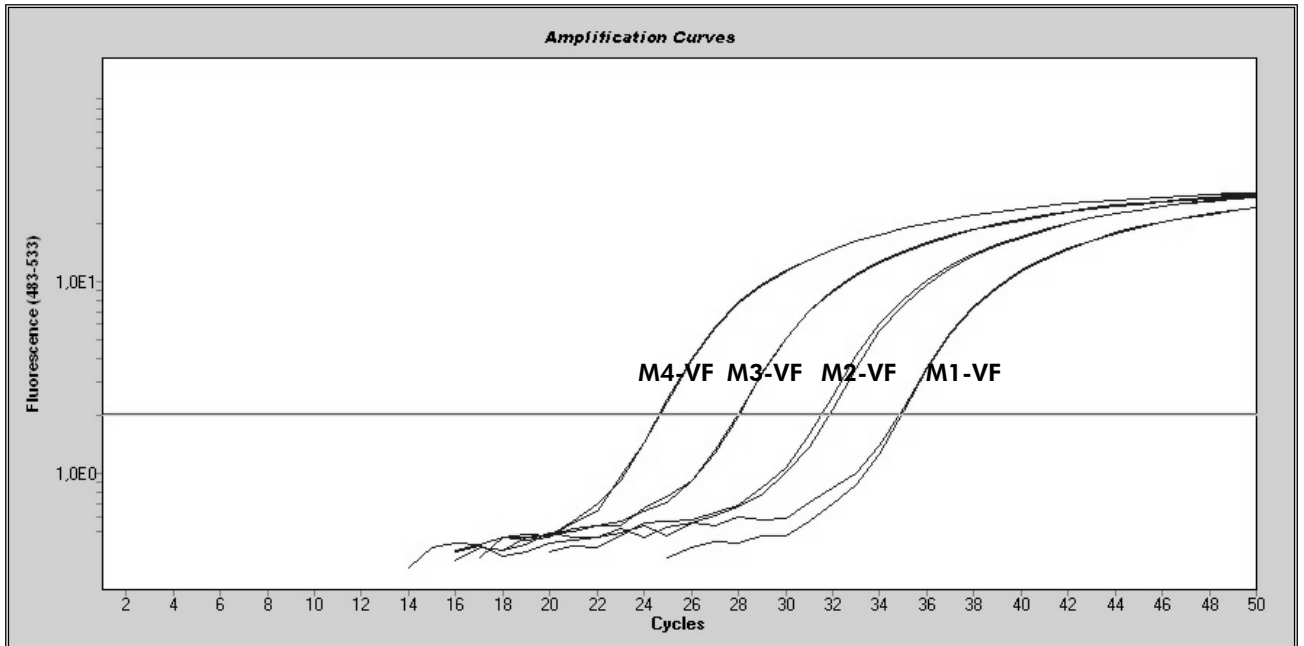
**Table 15. Quantitative data for the wild-type and V617F standards**

| Standard | Copy number (CN)   | $\log_{10} \text{CN}$ |
|----------|--------------------|-----------------------|
| M1-VF    | $5 \times 10^1$ VF | 1.7                   |
| M2-VF    | $5 \times 10^2$ VF | 2.7                   |
| M3-VF    | $5 \times 10^3$ VF | 3.7                   |
| M4-VF    | $5 \times 10^4$ VF | 4.7                   |
| WT-1     | $5 \times 10^1$ WT | 1.7                   |
| WT-2     | $5 \times 10^2$ WT | 2.7                   |
| WT-3     | $5 \times 10^3$ WT | 3.7                   |
| WT-4     | $5 \times 10^4$ WT | 4.7                   |

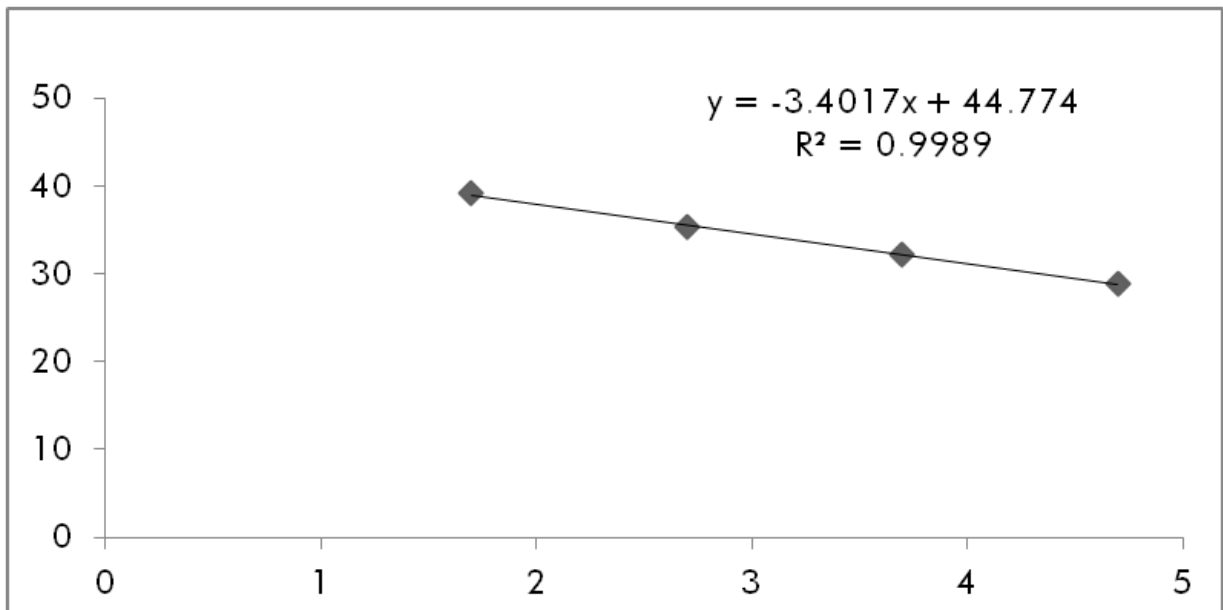
**Note:** Each user should measure their own reproducibility in their laboratory.

## Standard curve and quality criteria

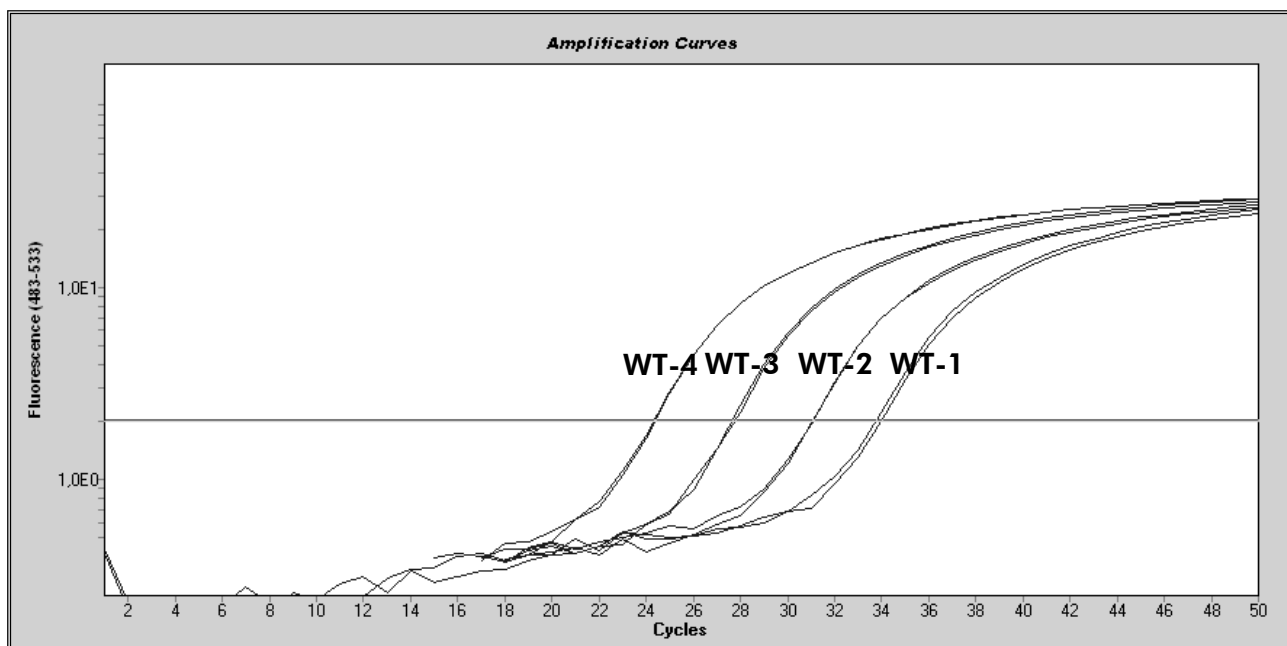
Figures 7 and 9 show examples of results obtained with *ipsogen* JAK2 MutaQuant Kit and Figures 8 and 10 show an example of the theoretical curve calculated on 4 standard dilutions.



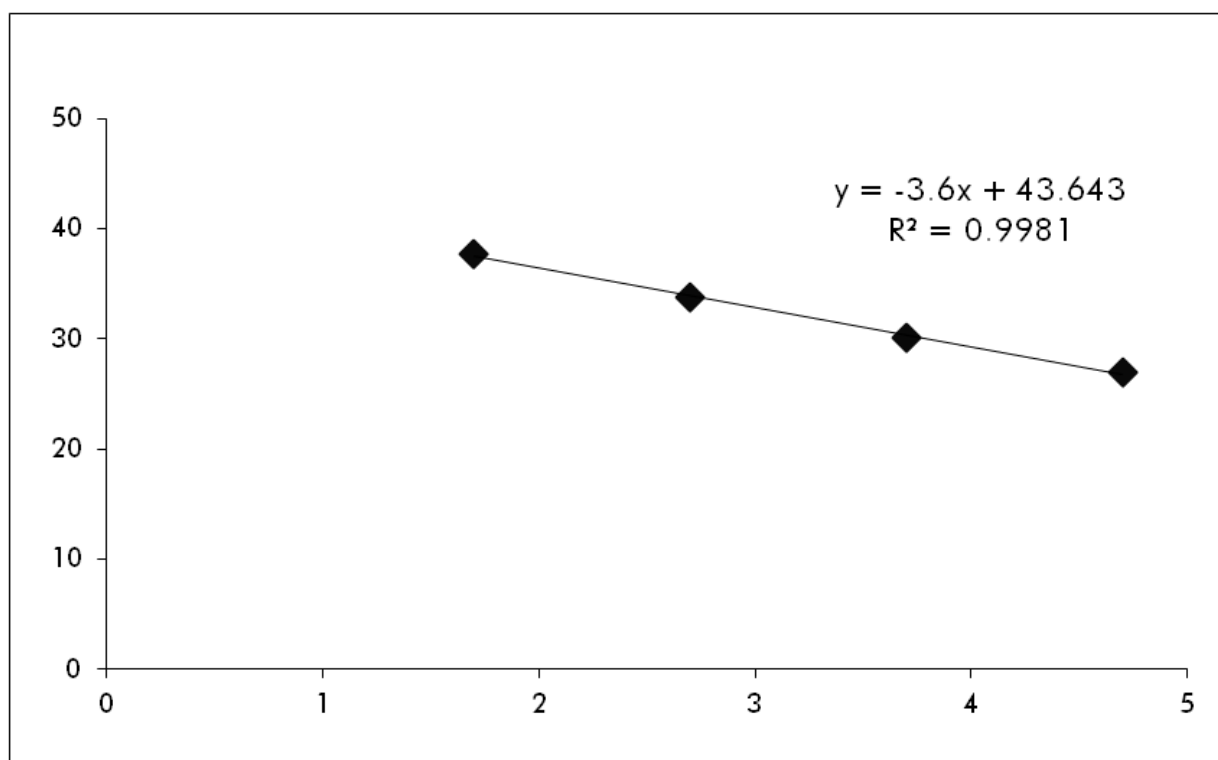
**Figure 7. Amplification plot of  $5 \times 10^1$ ,  $5 \times 10^2$ ,  $5 \times 10^3$ , and  $5 \times 10^4$  copies of the JAK2 V617F plasmid (controls M1-VF, M2-VF, M3-VF, M4-VF, respectively).**



**Figure 8. Standard curve for JAK2 V617F.**



**Figure 9. Amplification plot of  $5 \times 10^1$ ,  $5 \times 10^2$ ,  $5 \times 10^3$ , and  $5 \times 10^4$  copies of the JAK2 wild-type plasmid (controls WT-1, WT-2, WT-3, and WT-4, respectively).**



**Figure 10. Standard curve for JAK2 wild-type.**

As standards are 10-fold dilutions, the theoretical slope of the curve is  $-3.32$ . A slope between  $-3.0$  and  $-3.9$  is acceptable as long as  $R^2$  is  $>0.95$  (12). However, a value for  $R^2 >0.98$  is desirable for precise results (13).

The standard curve equations can then be used to calculate V617F and WT  $\log_{10}$  copy numbers in the unknown samples.

The V617F standard curve equation should be used to transform raw  $C_p/C_T$  value means (obtained with PPM-VF) for the unknown and control samples, into JAK2 V617F copy numbers ( $CN_{V617F}$ ).

$$\log_{10} CN_{V617F} = \frac{(\text{Mean } C_{pV617F} - \text{Standard curve intercept}_{V617F})}{\text{Standard curve slope}_{V617F}}$$

The wild-type standard curve equation should be used to transform raw mean  $C_p/C_T$  value (obtained with PPM-WT) for the unknown and control samples, into JAK2 wild-type copy numbers ( $CN_{WT}$ ).

$$\log_{10} CN_{WT} = \frac{(\text{Mean } C_{pWT} - \text{Standard curve intercept}_{WT})}{\text{Standard curve slope}_{WT}}$$

### Expression of the results

Results are relative to 25 ng of total genomic DNA and should be expressed as the percentage of JAK2 V617F as follows.

$$\text{JAK2 V617F \%} = \frac{CN_{V617F}}{(CN_{V617F} + CN_{WT})} \times 100$$

### Reproducibility between replicates

The data obtained should be consistent between duplicates.

### Positive and negative controls

The positive control or PC-VF should give a JAK2 V617F percentage that is higher than 99.9%.

The negative control or NC-VF should give a JAK2 V617F percentage that is lower than 0.1%.

If these controls fail to function correctly, please see the "Troubleshooting guide", page 33, to find a solution.



## Water controls

Negative controls should give zero CN for both the JAK2 V617F and JAK2 wild-type detection.

A positive water control results from a cross-contamination. See “Troubleshooting guide”, below, to find a solution.

## Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see “Contact Information”, page 42).

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### Comments and suggestions

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#### Standard curve for wild-type or V617F are not linear

Vial inversion, inversion during distribution, cross-contamination, partial degradation of the standard, RQPCR reagent, non-specific amplification, or PCR program error

Check pipetting scheme and the setup of the reaction.

Store the *ipsogen* JAK2 MutaQuant Kit at –15 to –30°C and keep primers and probe mixes) protected from light. See “Reagent Storage and Handling”, page 11.

Avoid repeated freezing and thawing.

#### No or low signal for one standard

Standard not distributed, or use of same PPM mix

Check pipetting scheme and the setup of the reaction.

Repeat the PCR run.

## Comments and suggestions

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### Negative (H<sub>2</sub>O) control is positive

Cross-contamination, reagent contamination, instrument error, well or capillary inversion, or probe degradation

Replace all critical reagents.

Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry over contamination.

Keep primers and probe mixes protected from light.

Check for false positives on fluorescence curves.

### No signal, even in standard control

a) Incorrect detection channel has been chosen

Set channel to F1/F2 or 530 nm/640 nm.

b) Pipetting error or omitted reagents

Check pipetting scheme and the setup of the reaction.

Repeat the PCR run.

c) No data acquisition program

Check the cycle program.

Select acquisition mode "Single" at the end of each annealing segment of the PCR program.

### Absent or low signal in samples but standard controls okay

Inhibitory effects of sample material caused by insufficient purification

Always check the DNA quality (OD<sub>260</sub>/OD<sub>280</sub>) and concentration before starting.

Repeat DNA preparation.

### Fluorescence intensity too low

a) Inappropriate storage of kit components

Aliquot reagents for storage.

Store the *ipsogen* JAK2 MutaQuant Kit at -15 to -30°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 11.

Avoid repeated freezing and thawing.

## Comments and suggestions

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b) Very low initial amount of target DNA

Check the amount of the sample DNA.

**Note:** Depending of the chosen method of DNA preparation, inhibitory effects may occur.

### Negative controls are positive

Carry over contamination

Replace all critical reagents.

Repeat the experiment with new aliquots of all reagents.

Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.

### Fluorescence intensity varies

a) Pipetting error

Vortex and spin all reagents after thawing.

LightCycler variability caused by so-called "pipetting error" can be reduced by analyzing data in the F1/F2 or 530 nm/640 nm mode.

b) Insufficient centrifugation of the plate, tubes, or capillaries, or the prepared PCR mix may still be in the upper vessel of the capillary, or an air bubble could be trapped in the capillary tip

Always centrifuge capillaries loaded with the reaction mix as described in the specific operating manual of the apparatus.

c) Outer surface of the capillary tip dirty

Always wear gloves when handling the capillaries.

## Comments and suggestions

---

### **Wild-type or V617F positive controls signal using the reciprocal PPM**

|  |   |
|--|---|
| Cross-contamination, reagent contamination, or well or capillary inversion | Replace all critical reagents.<br>Repeat the experiment with new aliquots of all reagents.<br>Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.<br>Check pipetting scheme and setup of the reaction. |
|--|---|

### **Inverted detection of positive control**

|  |   |
|--|---|
| Distributed inversion of PPM in well or capillary or in premix | Check pipetting scheme and setup of the reaction. |
|--|---|

### **No signal for one positive control or both**

|                            |   |
|----------------------------|---|
| PPM or control DNA omitted | Check pipetting scheme and setup of the reaction. |
|----------------------------|---|

### **High background**

|                       |  |
|-----------------------|--|
| Fluorophore bleaching | Store and handle probe protected from light. |
|-----------------------|--|

### **Poor reproducibility for the duplicate samples**

|  |   |
|--|---|
| Pipetting error or cross-contamination | Check pipetting scheme and the setup of the reaction. |
|--|---|

## Quality Control

Quality control of the complete kit has been performed on a LightCycler 480 instrument. This kit is manufactured according to ISO 13485:2003 standard. Certificates of analysis are available on request at [www.qiagen.com/support/](http://www.qiagen.com/support/).

## Limitations

The users must be trained and familiar with this technology prior the use of this device. This kit should be used following the instructions given in this manual, in combination with a validated instrument mentioned in "Materials Required but Not Provided", page 10.

Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings. 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.

Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

## **Performance Characteristics**

### **Nonclinical studies**

#### **Precision**

A precision study was conducted using 12 DNA samples extracted from cell lines that corresponded to different JAK2 V617F allele burdens. A total of 80 measurements were performed on each sample, using 3 different batches of the *ipsogen* JAK2 MutaQuant Kit. This precision study utilized an Applied Biosystems 7500 Real-Time PCR System.

Analytical data are summarized in Table 15.

**Table 15. Precision data DNA samples**

| Sample | Theoretical<br>JAK2 V617F<br>(%) | n* | Mean<br>(%) | CV<br>(%) | Percentile |         |
|--------|----------------------------------|----|-------------|-----------|------------|---------|
|        |                                  |    |             |           | 5          | 95      |
| A      | 0                                | 73 | 0.004       | 117.5     | 0.000      | 0.015   |
| B      | 0.05                             | 80 | 0.101       | 89.2      | 0.003      | 0.284   |
| C      | 0.5                              | 79 | 0.449       | 61.6      | 0.161      | 0.950   |
| D      | 1                                | 68 | 1.169       | 41.6      | 0.611      | 1.998   |
| E      | 2                                | 80 | 2.046       | 33.5      | 1.168      | 3.185   |
| F      | 4                                | 80 | 3.733       | 30.6      | 2.120      | 5.560   |
| G      | 5                                | 77 | 5.246       | 22.4      | 3.647      | 7.309   |
| H      | 12.5                             | 80 | 16.633      | 16.6      | 12.792     | 22.335  |
| I      | 31                               | 80 | 28.602      | 14.8      | 22.705     | 34.773  |
| J      | 50                               | 76 | 56.181      | 6.6       | 50.024     | 63.724  |
| K      | 78                               | 80 | 80.153      | 3.8       | 75.352     | 85.551  |
| L      | 100                              | 70 | 99.998      | 0.003     | 99.992     | 100.000 |

\* Outlying values were excluded. These were defined as values smaller than the lower quartile minus 3 times the interquartile range or larger than the upper quartile plus 3 times the interquartile range on a Box and Whisker plot.

n = number of validated samples; CV = global coefficient of variation.

### Limit of blank and limit of detection

The background level or level of blank (LOB) was determined on negative samples (8 samples, 76 measurements). This was found to be 0.014%.

The limit of detection (LOD) was determined using samples that were known to be positive but with low expression (7 samples, 68 measurements). This was found to be 0.061%, with a 90% confidence interval upper bound at 0.091%.

This optimal sensitivity can be obtained on specimens containing at least 10,000 copies of the JAK2 gene (wild-type or V617F mutation).

Quantification data should be reported as follows.

- JAK2 V617F  $\leq 0.014\%$  can be interpreted as the JAK2 V617F mutation was not detected.
- JAK2 V617F is  $>0.014\%$  but  $<0.091\%$  can be interpreted as an inconclusive result (gray zone).
- JAK2 V617F  $\geq 0.091\%$  can be interpreted as a positive result and that the JAK2 V617F mutation has been detected.

### **Linearity**

Linearity studies were performed on 12 samples, with each one obtained from a different mix of DNA extracted from cell lines that were positive and negative for the JAK2 V617F mutation. Each sample was tested 5 times. Data from this study showed that the *ipsogen JAK2 MutaQuant* Kit gave linear results across the dynamic range.

### **Clinical studies**

DNA from blood or bone marrow were extracted from 87 patient samples and analyzed using the *ipsogen JAK2 MutaQuant* Kit. In addition, the percentage of JAK2 V617F mutations were quantified and compared with screening test results obtained with the *ipsogen JAK2 MutaScreen EZ* Kit (catalog no. 673223). Data obtained are shown in Table 16.

**Table 16. Contingency table showing the agreement between results obtained with the *ipsogen* JAK2 MutaQuant Kit and the *ipsogen* JAK2 MutaScreen EZ Kit**

|  |                     | Results from <i>ipsogen</i><br>JAK2 MutaScreen EZ Kit |                     |                     | n  |
|--|---------------------|---|---------------------|---------------------|----|
|  |                     | Mutation detected                                     | Inconclusive result | Mutation undetected |    |
| Results from<br><i>ipsogen</i><br>JAK2<br>MutaQuant<br>Kit | Mutation detected   | 40  | 2                   | 7                   | 49 |
|  | Inconclusive result | 0   | 0                   | 21                  | 21 |
|  | Mutation undetected | 0   | 0                   | 17                  | 17 |
|  | n                   | 40  | 2                   | 45                  | 87 |
| Positive agreement   |                     | 100% (95% confidence interval: 91%, 100%)             |                     |                     |    |
| Negative agreement   |                     | 71% (95% confidence interval: 51%, 85%)               |                     |                     |    |
| Overall agreement  |                     | 89% (95% confidence interval: 79%, 95%)               |                     |                     |    |

## References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at [www.qiagen.com/RefDB/search.asp](http://www.qiagen.com/RefDB/search.asp) or contact QIAGEN Technical Services or your local distributor.



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## Symbols

The following symbols may appear on the packaging and labeling:











<N>

Contains reagents sufficient for <N> reactions



Use by

|   |                                    |
|---|------------------------------------|
|  | In vitro diagnostic medical device |
|  | Catalog number                     |
|  | Lot number                         |
|  | Material number                    |
|  | Global Trade Item Number           |
|  | Temperature limitation             |
|  | Manufacturer                       |
|  | Consult instructions for use       |

## Contact Information

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## Ordering Information

| Product   | Contents   | Cat. no. |
|---|--|----------|
| <i>ipsogen</i> JAK2<br>MutaQuant Kit (12)   | For 12 reactions: Wild-type JAK2 Gene Control, JAK2 V617F Control Gene, Primers and Probe Mix PPM-WT, Primers and Probe Mix PPM-VF   | 673522   |
| <i>ipsogen</i> JAK2<br>MutaQuant Kit (24)   | For 24 reactions: Wild-type JAK2 Gene Control, JAK2 V617F Control Gene, Primers and Probe Mix PPM-WT, Primers and Probe Mix PPM-VF   | 673523   |
| <b>Rotor-Gene Q MDx — for IVD-validated real-time PCR analysis in clinical applications</b> |  |          |
| Rotor-Gene Q MDx<br>5plex HRM Platform  | Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included | 9002032  |
| Rotor-Gene Q MDx<br>5plex HRM System  | Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training              | 9002033  |

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**www.qiagen.com**

**Australia** ■ techservice-au@qiagen.com

**Austria** ■ techservice-at@qiagen.com

**Belgium** ■ techservice-bnl@qiagen.com

**Brazil** ■ suportetecnico.brasil@qiagen.com

**Canada** ■ techservice-ca@qiagen.com

**China** ■ techservice-cn@qiagen.com

**Denmark** ■ techservice-nordic@qiagen.com

**Finland** ■ techservice-nordic@qiagen.com

**France** ■ techservice-fr@qiagen.com

**Germany** ■ techservice-de@qiagen.com

**Hong Kong** ■ techservice-hk@qiagen.com

**India** ■ techservice-india@qiagen.com

**Ireland** ■ techservice-uk@qiagen.com

**Italy** ■ techservice-it@qiagen.com

**Japan** ■ techservice-jp@qiagen.com

**Korea (South)** ■ techservice-kr@qiagen.com

**Luxembourg** ■ techservice-bnl@qiagen.com

**Mexico** ■ techservice-mx@qiagen.com

**The Netherlands** ■ techservice-bnl@qiagen.com

**Norway** ■ techservice-nordic@qiagen.com

**Singapore** ■ techservice-sg@qiagen.com

**Sweden** ■ techservice-nordic@qiagen.com

**Switzerland** ■ techservice-ch@qiagen.com

**UK** ■ techservice-uk@qiagen.com

**USA** ■ techservice-us@qiagen.com

