

ipsogen[®] JAK2 MutaSearch[®] Handbook



For JAK2 V617F/G1849T qualitative analysis.

For research use only. Not for use in diagnostic procedures.

For use with Rotor-Gene[®] Q, Applied Biosystems[®], ABI PRISM[®],
and LightCycler[®] instruments

REF 673813



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Intended Use

The *ipsogen* JAK2 MutaSearch Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The test can report false negative results in case of additional mutations located in nucleotides 88504 to 88622.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

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® 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.

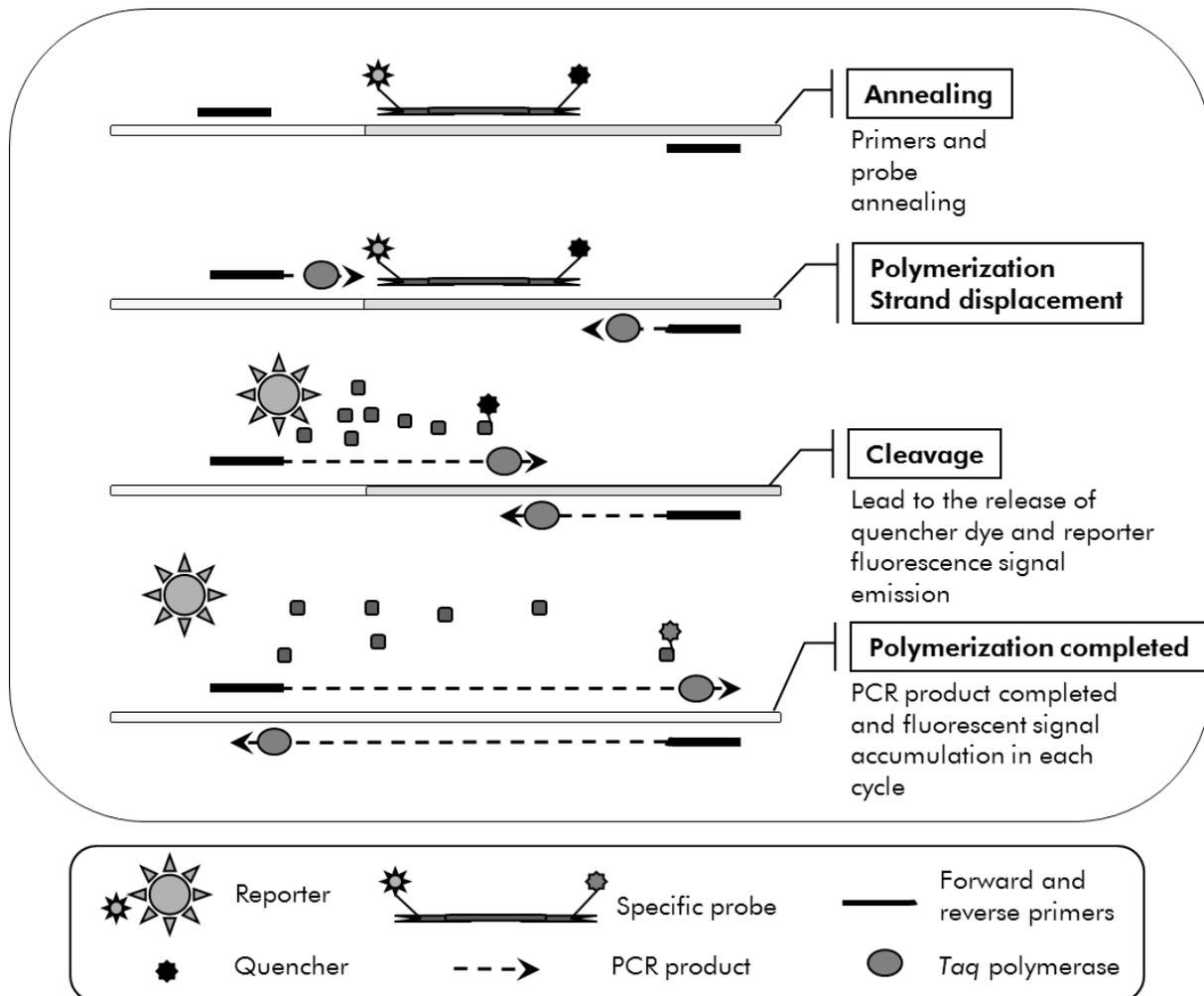


Figure 1. Reaction principle.

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

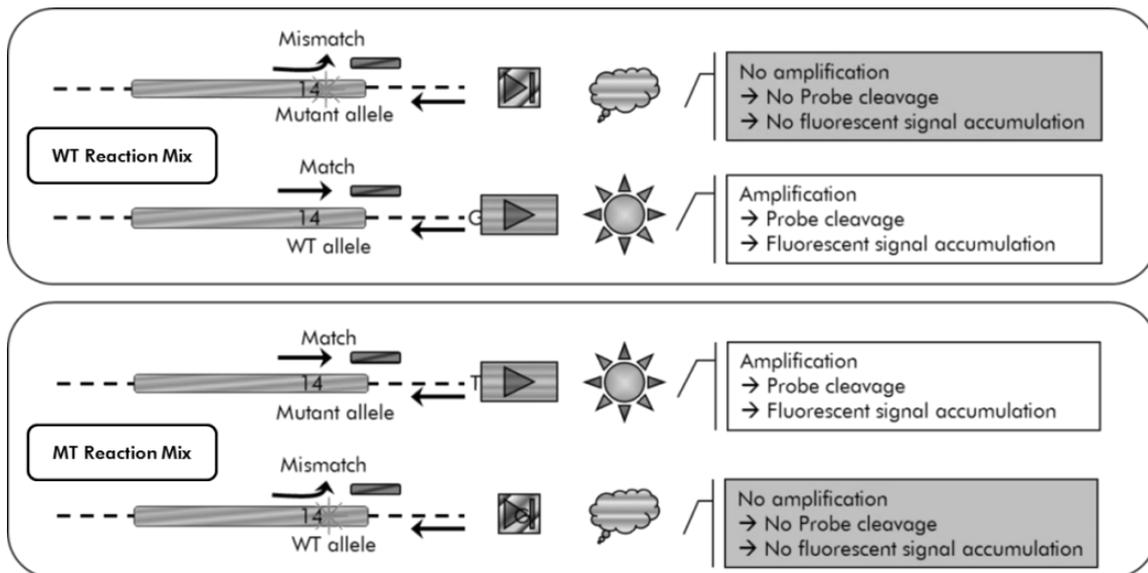


Figure 2. Allele specific PCR. Use of wild-type or the V617F primers and probe mix allows the specific detection of the wild-type or mutated allele in two separate reactions conducted using the same sample.

Materials Provided

Kit contents

<i>ipsogen JAK2 MutaSearch Kit</i>		(24)
Catalog no.		673813
Number of reactions		24
V617F Positive Control	PC-VF JAK2	40 μ l
V617F Negative Control	NC-VF JAK2	40 μ l
Cut-Off Sample (1% V617F allele)	COS-VF JAK2	40 μ l
Primers and probe mix JAK2 V617F*	PPM-JAK2 V617F 25x	68 μ l
Primers and probe mix JAK2 WT†	PPM-JAK2 WT 25x	68 μ l
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* Mix of specific reverse and forward primers for the *JAK2* gene, specific V617F FAM™–TAMRA™ probe.

† Mix of specific reverse and forward primers for the *JAK2* gene, specific wild-type FAM–TAMRA probe.

Note: Briefly centrifuge tubes before use.

Note: Analyzing unknown samples with the *ipsogen JAK2 MutaSearch Kit* requires the extraction of genomic DNA. Reagents needed to perform DNA extraction are not provided and must be validated in combination with the kit.

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
- Nuclease-free 1x TE buffer, pH 8.0
- Buffer and Taq DNA polymerase: The recommended reagents are TaqMan[®] Universal PCR Master Mix (Master Mix PCR 2x) (Thermo Fisher Scientific Inc., cat. no. 4304437) and LightCycler TaqMan Master (Master Mix PCR 5x) (Roche, cat. no. 04535286001) or LightCycler FastStart DNA Master^{PLUS} HybProbe[®] (Master Mix 5x) (Roche, cat. no. 03515567001).
Note: this Master Mix can only be used for LightCycler 1.2)
- Reagents for 0.8–1% agarose gel in 0.5x TBE electrophoresis buffer

Consumables

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

Equipment

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

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

Warnings and Precautions

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.

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

General precautions

qPCR tests require good laboratory practices, including equipment maintenance, that are dedicated to molecular biology and compliant with applicable regulations and relevant standards.

This kit is intended for research use. Reagents and instructions supplied in this kit have been tested for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. PPM-JAK2 reagents may be altered if exposed to light. All reagents are formulated specifically for use with this kit. For optimal performance of the kit, no substitutions should be made.

Use extreme caution to prevent:

- DNase contamination which 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, PCR product) 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 -15°C to -30°C upon receipt.

- Minimize exposure to light of the primers and probe mixes (PPM-JAK2 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, polynuclear cells, or granulocytes. To be able to compare results, we recommend adopting the same cellular fraction and DNA extraction method. DNA extraction should be performed by any home brew or commercial method.

DNA quantity is determined by measuring optical density at 260 nm. DNA quality should be assessed by spectrophotometry or gel electrophoresis.

The A_{260}/A_{280} ratio should be 1.7–1.9. Smaller ratios usually indicate contamination by protein or organic chemicals. Electrophoretic analysis on a 0.8–1% agarose gel should allow visualization of the isolated DNA as a distinct band of about 20 kb. A slight smear is acceptable.

The resultant DNA is diluted to 5 ng/ μ l in TE buffer. The qPCR reaction is optimized for 25 ng of purified genomic DNA.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing purified nucleic acids at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –20°C.

Protocol: qPCR on the Rotor-Gene Q 5plex HRM instrument with 72-tube rotor

Using this instrument, we recommend performing all measurements in duplicate, as indicated in Table 1.

Table 1. Number of reactions for the Rotor-Gene Q instrument with 72-tube rotor

Samples	Reactions
With the JAK2 V617F primers and probe mix (PPM-JAK2 V617F)	
n DNA samples	n x 2 reactions
3 DNA controls	6 reactions (PC-VF, NC-VF, and COS-VF, each one tested in duplicate)
Water control	2 reactions
With the JAK2 WT primers and probe mix (PPM-JAK2 WT)	
n DNA samples	n x 2 reactions
3 DNA controls	6 reactions (PC-VF, NC-VF, and COS-VF, each one tested in duplicate)
Water control	2 reactions

Sample processing on the Rotor-Gene Q instrument with 72-tube rotor

We recommend testing at least 12 DNA samples in the same experiment to optimize the use of the controls and primers and probes mixes. The rotor scheme in Figure 3 shows an example of such an experiment.

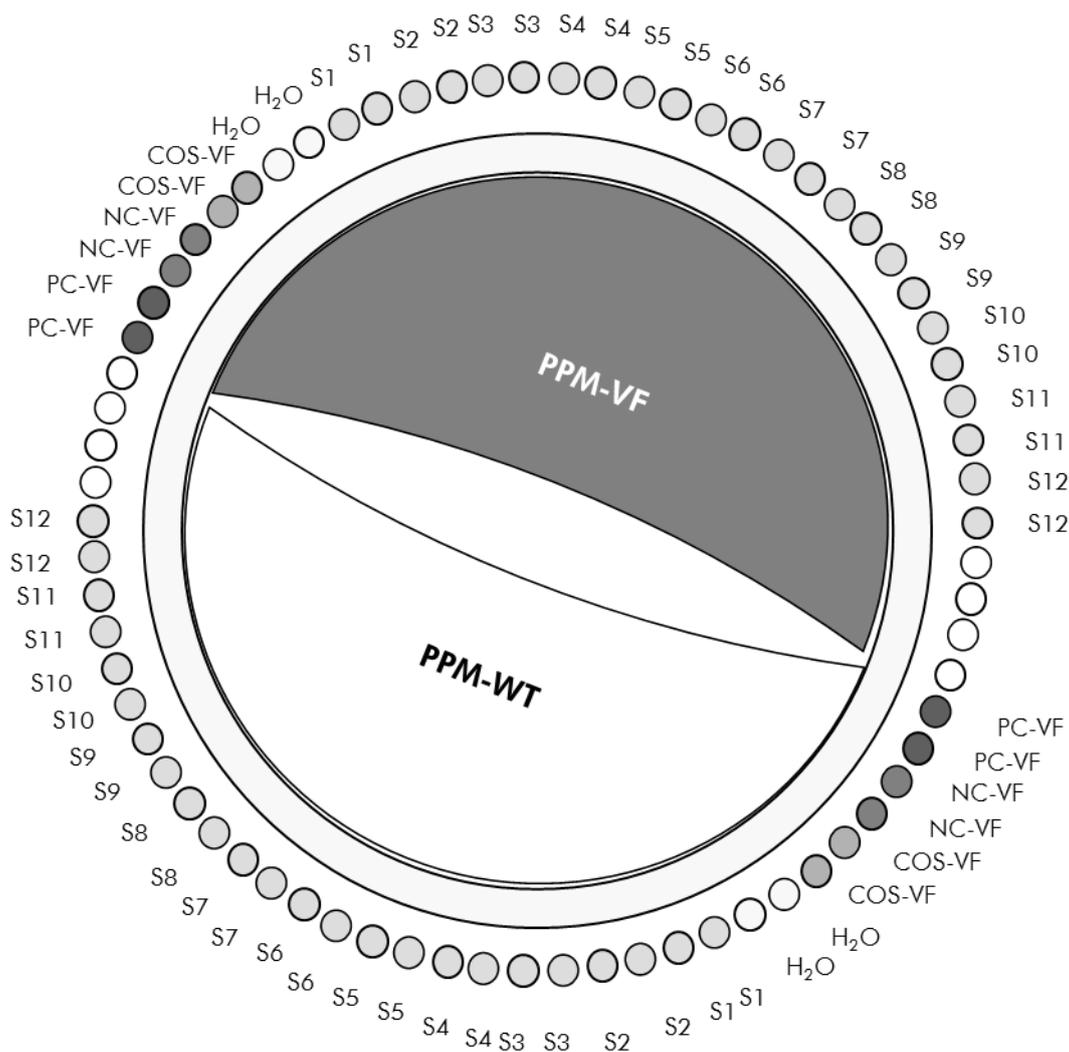


Figure 3. Suggested rotor setup for an experiment with the *ipsogen* JAK2 MutaSearch Kit. PC-VF: positive control; NC-VF: negative control; COS-VF: cut-off sample; S: DNA sample; H₂O: water control.

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 the Rotor-Gene Q instrument with 72-tube rotor

Note: Perform all steps on ice.

Procedure

1. Thaw all necessary components and place them on ice.

Components should be taken out of the freezer approximately 10 min before starting the procedure.

2. **Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
3. **Prepare the following qPCR mixes according to the number of samples being processed.**

All concentrations are for the final volume of the reaction.

Table 2 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 μ l. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix. Extra volumes are included to compensate for pipetting error.

Table 2. Preparation of qPCR mixes

Component	1 reaction (μl)	VF: 32+1 reactions (μl)	WT: 32+1 reactions (μl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	412.5	412.5	1x
Primers and probe mix, 25x (VF or WT, respectively)	1	33	33	1x
Nuclease- free PCR grade water	6.5	214.5	214.5	–
Sample (to be added at step 6)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

4. **Vortex and briefly centrifuge each qPCR mix (VF and WT) (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
5. **Dispense 20 μ l of the respective qPCR pre-mix (VF or WT) per tube.**
6. **Add 5 μ l of the sample DNA material or controls in the corresponding tube (total volume 25 μ l).**
7. **Mix gently, by pipetting up and down.**

8. Close the PCR tubes. Place the tubes in the 72-tube rotor according to the manufacturer's recommendations. Fill all other positions with empty tubes.
9. Program the Rotor-Gene Q instrument with the thermal cycling program as indicated in Table 3.

Table 3. Temperature profile

Mode of analysis	Quantitation
Hold	Temperature: 50 deg Time: 2 mins
Hold 2	Temperature: 95 deg Time: 10 mins
Cycling	50 times 95 deg for 15 secs 62 deg for 1 min with acquisition of FAM fluorescence in channel Green: Single

10. For the Rotor-Gene Q instrument, select "Slope Correct" for the analysis. We recommend setting the threshold at 0.03. Start the thermal cycling program, as indicated in Table 3.

Protocol: qPCR on Applied Biosystems7500, ABI PRISM 7900HT, or LightCycler 480 instruments

Using 96-well plate qPCR equipment, we recommend performing all measurements in duplicate as indicated in Table 4.

Table 4. Number of reactions for Applied Biosystems 7500, ABI PRISM 7900HT, or LightCycler 480 instruments

Samples	Reactions
With the JAK2 V617F primers and probe mix (PPM-JAK2 V617F)	
n DNA samples	n x 2 reactions
3 DNA controls	6 reactions (PC-VF, NC-VF, and COS-VF, each one tested in duplicate)
Water control	2 reactions
With the JAK2 WT primers and probe mix (PPM-JAK2 WT)	
n DNA samples	n x 2 reactions
3 DNA controls	6 reactions (PC-VF, NC-VF, and COS-VF, each one tested in duplicate)
Water control	2 reactions

Sample processing on Applied Biosystems 7500, ABI PRISM 7900HT, or LightCycler 480 instruments

We recommend testing at least 12 DNA samples in the same experiment to optimize the use of the controls and primers and probe mixes. The plate scheme in Figure 4 shows an example of such an experiment.

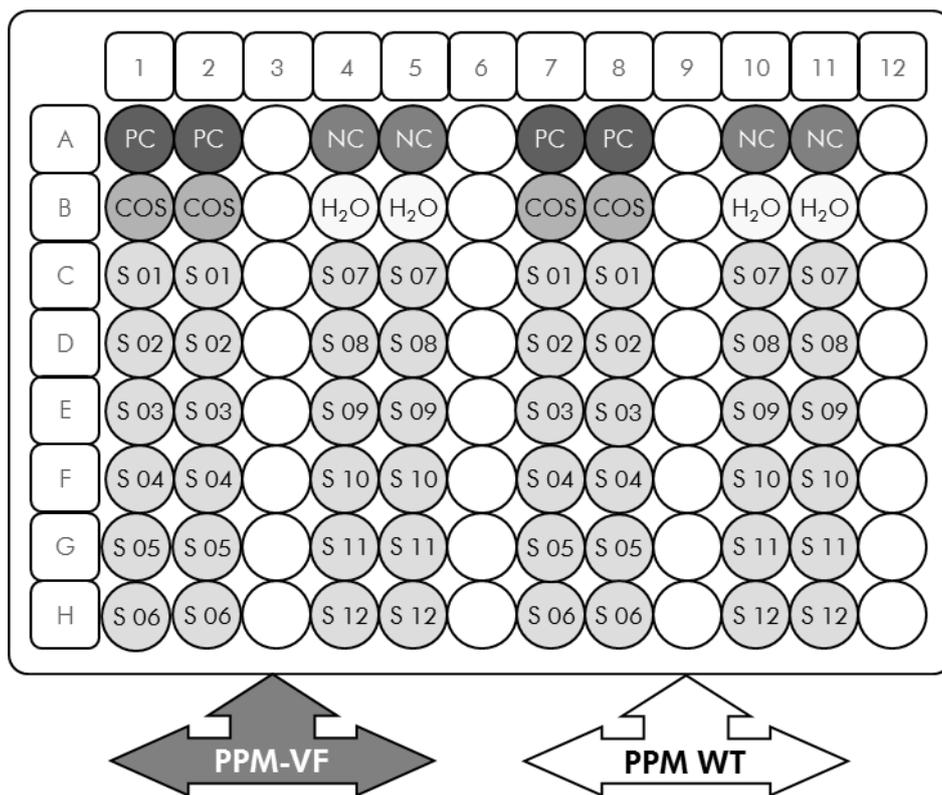


Figure 4. Suggested plate setup for an experiment with the *ipsogen* JAK2 MutaSearch Kit. PC: positive control; NC: negative control; COS: cut-off sample; S: DNA sample; H₂O: water control.

qPCR on Applied Biosystems 7500, ABI PRISM 7900HT, or LightCycler 480 instruments

Note: Perform all steps on ice.

Procedure

- 1. Thaw all necessary components and place them on ice.**
Components should be taken out of the freezer approximately 10 min before starting the procedure.
- 2. Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
- 3. Prepare the following qPCR mix according to the number of samples being processed.**

All concentrations are for the final volume of the reaction.

Table 5 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 μ l. A pre-mix can be prepared, according to the number of reactions, using the same primers and probe mix. Extra volumes are included to compensate for pipetting error.

Table 5. Preparation of qPCR mix

Component	1 reaction (μl)	VF: 32+1 reactions (μl)	WT: 32+1 reactions (μl)	Final concentration
TaqMan Universal PCR Master Mix, 2x	12.5	412.5	412.5	1x
Primers and probe mix, 25x (VF or WT, respectively)	1	33	33	1x
Nuclease- free PCR grade water	6.5	214.5	214.5	–
Sample (to be added at step 6)	5	5 each	5 each	–
Total volume	25	25 each	25 each	–

- 4. Vortex and briefly centrifuge each qPCR mix (VF and WT) (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
- 5. Dispense 20 μ l of the respective qPCR pre-mix (VF or WT) per well.**
- 6. Add 5 μ l of the sample DNA material or controls in the corresponding well (total volume 25 μ l).**
- 7. Mix gently, by pipetting up and down.**
- 8. Close the plate and briefly centrifuge (300 x g, approximately 10 s).**
- 9. Place the plate in the thermal cycler according to the manufacturer's recommendations.**
- 10. Program the thermal cycler with the thermal cycling program as indicated in Table 6 for Applied Biosystems 7500 and ABI PRISM 7900HT SDS, or Table 8 for the LightCycler 480 instrument.**

Table 6. Temperature profile for Applied Biosystems 7500 and ABI PRISM 7900HT SDS

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

Table 7. Temperature profile for the LightCycler 480 instrument

Mode of analysis	Absolute Quantification (“Abs Quant”)
Detection formats	Select “Simple Probe” in the Detection formats window
Hold	Temperature: 50°C Time: 2 minutes
Hold 2	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 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: Single

11. For the Applied Biosystems 7500 and ABI PRISM 7900HT SDS, follow step 11a. For the LightCycler 480 instrument, follow step 11b.

11a. Applied Biosystems 7500 and ABI PRISM 7900HT SDS: We recommend a threshold set at 0.1 in the analysis step. Start the cycling program, as indicated in Table 6.

11b. LightCycler 480 instrument: 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 7.

Protocol: qPCR on the LightCycler 1.2 instrument

Using capillary instruments, we recommend measuring samples in duplicate and controls only once, as indicated in Table 8.

Table 8. Number of reactions for the LightCycler 1.2 instrument

Samples	Reactions
With the JAK2 V617F primers and probe mix (PPM-JAK2 V617F)	
n DNA samples	n x 2 reactions
3 DNA controls	3 reactions (PC-VF, NC-VF, and COS-VF, each one tested once)
Water control	1 reaction
With the JAK2 WT primers and probe mix (PPM-JAK2 WT)	
n DNA samples	n x 2 reactions
3 DNA controls	3 reactions (PC-VF, NC-VF, and COS-VF, each one tested once)
Water control	1 reaction

Sample processing on the LightCycler 1.2 instrument

We recommend testing six DNA samples in the same experiment to optimize the use of the controls and primers and probe mixes. The capillary scheme in Figure 5 shows an example of such an experiment.

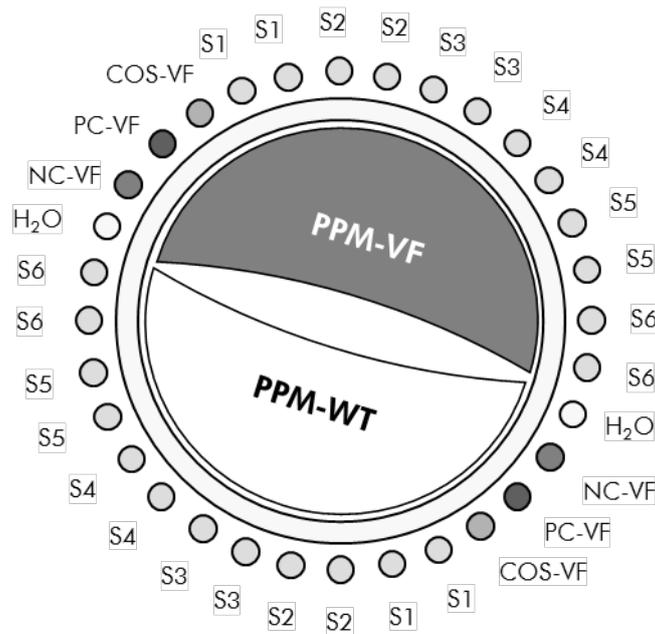


Figure 5. Suggested rotor setup for an experiment with the ipsogen JAK2 MutaSearch Kit. PC-VF: positive control; NC-VF: negative control; COS: cut-off sample; S: DNA sample; H₂O: water control.

qPCR on the LightCycler 1.2 instrument

Note: Because of particular technological requirements, LightCycler 1.2 experiments must be performed using specific reagents. We recommend to use the LightCycler FastStart DNA Master^{PLUS} HybProbe and to follow 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.

Components should be taken out of the freezer approximately 10 min before starting the procedure.

2. Vortex and briefly centrifuge all the tubes (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).

3. Prepare the following qPCR mix according to the number of samples being processed.

All concentrations are for the final volume of the reaction.

Table 9 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 20 μ l. A pre-mix can be prepared, according to the number of reactions, using the same primer and probe mix. Extra volumes are included to compensate for pipetting error.

Table 9. Preparation of qPCR mix

Component	1 reaction (μl)	VF: 16+1 reactions (μl)	WT: 16+1 reactions (μl)	Final concentration
LightCycler FastStart DNA Master ^{PLUS} HybProbe, 5x	4	68	68	1x
Primers and probe mix, 25x (VF or WT, respectively)	0.8	13.6	13.6	1x
Nuclease- free PCR grade water	10.2	173.4	173.4	–
Sample (to be added at step 6)	5	5 each	5 each	–
Total volume	20	20 each	20 each	–

4. **Vortex and briefly centrifuge each qPCR mix (VF and WT) (approximately 10 s, 10,000 rpm, to collect the liquid in the bottom of the tube).**
5. **Dispense 15 μ l of the respective qPCR pre-mix (VF or WT) per capillary.**
6. **Add 5 μ l of the sample DNA material or controls in the corresponding capillary (total volume 20 μ l).**
7. **Mix gently, by pipetting up and down.**
8. **Close the capillaries and briefly centrifuge (500 x g, approximately 5 s).**
9. **Load the capillaries into the thermal cycler according to the manufacturer's recommendations.**
10. **Program the LightCycler 1.2 instrument with the thermal cycling program as indicated in Table 10.**

Table 10. Temperature profile

Mode of analysis	Quantification
Hold	Temperature: 95°C Time: 10 minutes
Cycling	50 times 95°C for 15 seconds 66°C for 1 minute; with acquisition of FAM fluorescence: Single

11. For the LightCycler 1.2, the F1/F2 and “2nd derivative analysis” mode is recommended. Start the thermal cycling program, as indicated in Table 10.

Results

$\Delta\Delta C_T$ (or $\Delta\Delta C_p$) calculation and genotyping

Extract exported data from the Analyze Export File generated by the system, and analyze results as described below.

Note: C_T values are results obtained from Rotor-Gene, Applied Biosystems, and ABI PRISM systems. C_p values, obtained from LightCycler systems, may be substituted for C_T values in the description below. Calculations are presented for C_T values and may be applied to C_p values in the same way.

IMPORTANT: If no amplification is observed (i.e., “undetected”, $C_T > 45$, or $C_p > 45$, depending on the instrument used) for both PPM-JAK2 WT and PPM-JAK2 VF, the results cannot be analyzed. These results indicate that the concentration of DNA in the sample was not within the acceptable range or that the DNA matrix was omitted. Otherwise, proceed with analysis as described below.

Procedure

- 1. Calculate the mean C_T value obtained with PPM-JAK2 V617F (Mean C_T VF) and PPM-JAK2 WT (Mean C_T WT) for each sample (controls, cut-off sample, and unknown samples).**

If one of the duplicates for a sample has an “undetermined” value, do not take it into account: use only the value obtained for the other duplicate. In this case, we strongly recommend retesting the sample.

If both duplicates are undetermined, set the sample value to 45.

- 2. Calculate the input limit (IL) according to the scheme below.**

Input limit (IL) = Mean C_T WT for COS + 3.3

Note: The input limit enables checking that the DNA sample used for the test has been correctly handled, so as to guarantee the final JAK2 V617F status results obtained.

3. Check sample quality for each unknown sample according to Table 11.

Table 11. Criteria for sample quality

If:	Then:
Mean C_T VF < 40	Proceed with step 4.
Mean C_T VF \geq 40 and Mean C_T WT < IL	Proceed with step 4.
Mean C_T VF \geq 40 and Mean C_T WT \geq IL	Sample cannot be analyzed.*

* Concentration of DNA in the sample was not within the acceptable range or the DNA matrix was omitted.

4. Calculate the ΔC_T value for all valid samples ($\Delta C_{T \text{ Sample}}$) and controls ($\Delta C_{T \text{ PC-VF}}$, $\Delta C_{T \text{ NC-VF}}$ and $\Delta C_{T \text{ COS}}$) according to the scheme below.

$$\Delta C_T = \text{Mean } C_T \text{ VF} - \text{Mean } C_T \text{ WT}$$

5. Calculate the $\Delta \Delta C_T$ value for each unknown sample ($\Delta \Delta C_{T \text{ Sample}}$) and for each control ($\Delta \Delta C_{T \text{ PC-VF}}$ and ($\Delta \Delta C_{T \text{ NC-VF}}$) according to the schemes below.

$$\Delta \Delta C_{T \text{ Sample}} = \Delta C_{T \text{ COS}} - \Delta C_{T \text{ Sample}}$$

$$\Delta \Delta C_{T \text{ PC-VF}} = \Delta C_{T \text{ COS}} - \Delta C_{T \text{ PC-VF}}$$

$$\Delta \Delta C_{T \text{ NC-VF}} = \Delta C_{T \text{ COS}} - \Delta C_{T \text{ NC-VF}}$$

6. Calculate the gray zone, or the incertitude area, around the COS-VF according to the scheme below.

Note: The gray zone (GZ) of a test is defined as an area of values where the discriminatory performance is insufficiently accurate. A value in the gray zone indicates that the target marker cannot be scored as either present or absent. The gray zone must be calculated for each experiment. Based on variations observed during assay precision studies GZ has been defined as $\pm 7\%$ of the $\Delta C_{T \text{ COS}}$.

This calculation is valid for all the experiments and on all the recommended instruments.

$$\text{GZ: } [(-\Delta C_{T \text{ COS}} \times 0.07); (+\Delta C_{T \text{ COS}} \times 0.07)]$$

7. Determine the genotype of unknown samples according to Table 12.

Table 13 gives an example of calculations and interpretation of results for a representative experiment.

Table 12. Interpretation of genotyping results

Results	Interpretation
$\Delta\Delta C_{T \text{ Sample}} > +\Delta C_{T \text{ COS}} \times 0.07$	JAK2 V617F mutation is detected.
$\Delta\Delta C_{T \text{ Sample}} < -\Delta C_{T \text{ COS}} \times 0.07$	JAK2 V617F mutation is not detected.
$\Delta\Delta C_{T \text{ Sample}}$ within GZ $(-\Delta C_{T \text{ COS}} \times 0.07 \leq \Delta\Delta C_{T \text{ Sample}} \leq +\Delta C_{T \text{ COS}} \times 0.07)$	Result inconclusive.

Table 13. Example of calculations and interpretation of results for a representative experiment

Sample	C_T VF	Mean C_T VF	C_T WT	Mean C_T WT	ΔC_T	$\Delta\Delta C_T$	Evaluation
PC	27.82	27.74	40.27	40.24	-12.50	20.12	Positive
PC	27.66		40.20				
NC	41.23	41.10	26.66	26.76	14.34	-6.72	Negative
NC	40.96		26.85				
COS	35.04	34.85	27.28	27.23	7.62	0	IL = 30.53 GZ: -0.53 to +0.53
COS	34.66		27.17				
Sample 1	42.15	41.63	28.86	28.80	12.83	-5.21	Negative
Sample 1	41.10		28.73				
Sample 2	30.54	30.73	28.99	29.10	1.63	5.99	Positive
Sample 2	30.92		29.20				
Sample 3	37.31	37.71	30.11	30.22	7.49	0.13	Inconclusive (within GZ)
Sample 3	38.11		30.33				
Sample 4	45	45	39.25	38.85	Cannot be analyzed (Mean C_T VF >40 and Mean C_T WT >IL)		
Sample 4	45		38.45				

Controls

The water control should give no C_T (or C_p) value, both with JAK2 V617F and with JAK2 WT. A C_T (C_p) value for a water control may indicate cross-contamination. See "Troubleshooting guide", below.

The PC-VF should be interpreted as a sample for which the JAK2 V617F mutation is detected.

NC-VF should be interpreted as a sample for which the JAK2 V617F mutation is not detected.

See "Troubleshooting guide", below, for interpretation of inappropriate results.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see "Contact Information", page 32).

Comments and suggestions

Positive control signal negative

- | | |
|--|---|
| a) Pipetting error | Check pipetting scheme and the setup of the reaction.

Repeat the PCR run. |
| b) Inappropriate storage of kit components | Store the <i>ipsogen</i> JAK2 MutaSearch Kit at -15 to -30°C and keep primers and probe mix (PPM) protected from light. See "Reagent Storage and Handling", page 10.

Avoid repeated freezing and thawing.

Aliquot reagents for storage. |

Comments and suggestions

Negative controls are positive or positive controls are positive with the wrong PPM

- | | |
|---------------------|--|
| Cross-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. |
|---------------------|--|

No signal, even in positive controls

- | | |
|---|--|
| a) Pipetting error or omitted reagents | Check pipetting scheme and the setup of the reaction.
Repeat the PCR run. |
| b) Inhibitory effects of the sample material, caused by insufficient purification | Repeat the DNA preparation. |
| c) LightCycler: Incorrect detection channel chosen | Set Channel Setting to F1/F2 or 530 nm/640 nm. |
| d) LightCycler: No data acquisition programmed | Check the cycle programs.
Select acquisition mode "single" at the end of each annealing segment of the PCR program. |

Absent or low signal in samples but positive controls okay

- | | |
|---------------------------------------|---|
| Poor DNA quality or low concentration | Always check the DNA quality and concentration before starting. |
|---------------------------------------|---|

LightCycler: Fluorescence intensity too low

- | | |
|--|---|
| a) Inappropriate storage of kit components | Store the <i>ipsogen</i> JAK2 MutaSearch Kit at –15 to –30°C and keep primers and probe mix (PPM) protected from light. See "Reagent Storage and Handling", page 10.
Avoid repeated freezing and thawing.
Aliquot reagents for storage. |
|--|---|

Comments and suggestions

- b) Very low initial amount of target DNA Increase the amount of sample DNA.
Note: Depending of the chosen method of DNA preparation, inhibitory effects may occur.

LightCycler: Fluorescence intensity varies

- a) Pipetting error 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 capillaries 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *ipsogen*[®] JAK2 MutaSearch Kit is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request at www.qiagen.com/support/.

Symbols

The following symbols may appear on the packaging and labeling:



<N>

Contains reagents sufficient for <N> reactions



Use by



Catalog number



Lot number



Material number



Global Trade Item Number



Temperature limitation



Manufacturer



Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
<i>ipsogen</i> JAK2 MutaSearch Kit (24)	For 24 reactions: V617F Positive Control, V617F Negative Control, Cut-Off Sample, Primers and Probe Mix JAK2 wild-type and JAK2 V617F	673813
Rotor-Gene Q – for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler 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	9001580
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler 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	9001650
QIAamp® DNA Blood Maxi Kit – for purification of genomic DNA from blood		
QIAamp DNA Blood Maxi Kit (10)	For 10 DNA maxipreps: 10 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 ml)	51192
QIAamp DNA Blood Maxi Kit (50)	For 50 DNA maxipreps: 50 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 ml)	51194

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Notes

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