

Quantiplus® JAK-2 (V617F) Mutation Detection Real-Time PCR Kit



QL-JAK-25 : 25 rxns
QL-JAK-50 : 50 rxns
QL-JAK-100 : 100 rxns

IVD

PI/QLJAK-03

Intended Use

Quantiplus® JAK-2 (V617F) Mutation Detection Real-Time PCR Kit is used to detect specific V617F mutation of JAK-2 in blood and bone marrow using Real-Time qualitative PCR. The kit detects JAK-2G1849T (V617F) mutation in genomic DNA from subjects with suspected myeloproliferative neoplasm. The kit contains Amplification mix with specific Primers and Probes, Positive Control, and Negative Control.

Background Information

Janus kinase 2 (commonly called JAK-2) is a non-receptor tyrosine kinase. It is a member of the Janus kinase family and implicated in signaling by members of the type II cytokine receptor family (e.g. Interferon receptors). JAK-2 signaling is activated downstream from the prolactin receptor. This assay detects V617F mutation in JAK-2, the mutation corresponds to a single-nucleotide change of JAK-2 nucleotide 1849 in exon 14 of JAK-2. Several research findings have established an association between changes in the JAK-2 gene and several Myeloproliferative Disorders (MPDs).

Kit Components

| Color Coding (Caps) | Contents | Description | 25rxns (QL-JAK-25) | 50rxns (QL-JAK-50) | 100rxns (QL-JAK-100) |
|---------------------|----------------|--|--------------------|--------------------|----------------------|
| Amber | JAK2 Ready Mix | JAK-2 and Internal Control Probes and Primers along with Amplification Mix | 1 X 375 µL | 1 X 750 µL | 2x750µL |
| Red | JAK2 PC | JAK- 2 Positive Control | 1 x 100 µL | 1 x 100 µL | 2 x 100 µL |
| Natural | JAK2 NC | JAK-2 Negative Control | 1 x 100 µL | 1 x 100 µL | 2 x 100 µL |
| White | Huwel PW | Purified Water | 1 x 500 µL | 1 x 500 µL | 2 x 500 µL |

Note: Please pay attention to the cap color coding and the tube contents.

Huwel PW: Molecular Biology Grade Purified Water

Storage and Transportation Conditions

The kits should be transported at temperature below –20 °C. The kit is stable until the expiry date printed on the package, if the storage temperature is within –20 ±5 °C. More than 4X freezing and thawing cycles reduce the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical specifications

| | |
|-----------------------------|--|
| Target | Exon 14- V617F mutation in Janus Kinase gene |
| Specificity | 100% |
| Limit of Detection | ≤ 1% JAK-2 Mutation |
| Validated Specimen | Blood EDTA |
| External Quality Assessment | QCMD EQA Panels |

Assay Procedure

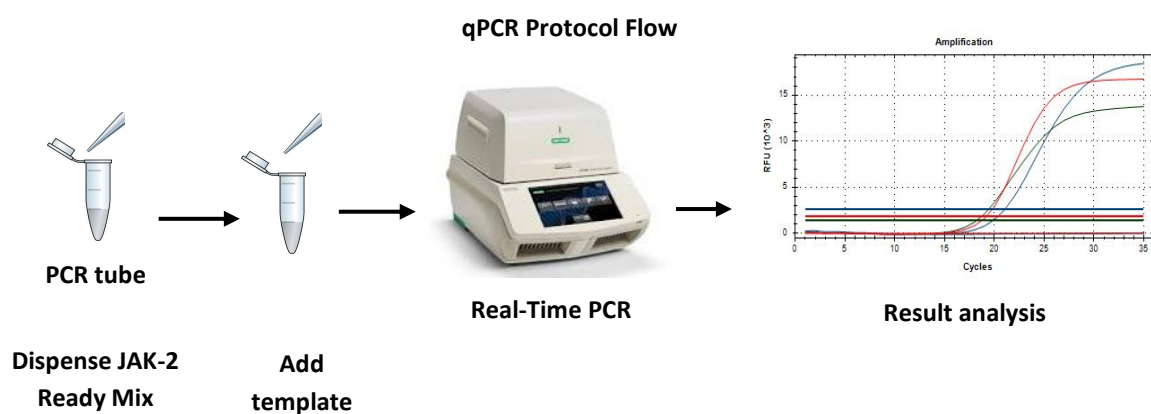
DNA Extraction

Quantiplus® JAK-2 (V617F) Mutation Detection Real-Time PCR Kit has been validated using the Genomic DNA extraction kits mentioned below. Recommended sample volume for extraction and elution are as follows:

| S. No. | Name of the Extraction Kit | Recommended Sample volume for Extraction | Recommended Final Elution volume |
|--------|---|--|----------------------------------|
| 1. | Huwel Genomic DNA Extraction Kit (Cat. No. HL-GDX-100) | 1mL | 100 µL |
| 2. | Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100) | 200 µL | 100 µL |
| 3. | QIAamp® DNA Blood Mini Kit (Cat. No. 51104) | 200 µL | 100 µL |

Note: Customer can also validate their own extraction process using other Genomic DNA extraction Kits.

**Note: For optimal assay performance 38-42 ng of DNA /PCR is recommended for clinical samples.*



Preparation of Reaction Master Mix

| Components | Volume per reaction (for 25µL) |
|---|--------------------------------|
| JAK2 Ready Mix | 15.0 |
| Extracted DNA/ JAK2 PC/ JAK2 NC/ Huwel PW | 10.0 |

JAK-2 NC: DNA with the wild type JAK-2 allele is provided as a negative control

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycler.

Cycling Conditions

| Steps | No. of cycles | Temperature (°C) | Time |
|--------------------------|---------------|------------------|---------|
| 1 (Initial denaturation) | 1 | 95 | 15 min. |
| 2 (PCR cycling) | 45 | 95 | 15 sec. |
| | | 60* | 1 min. |

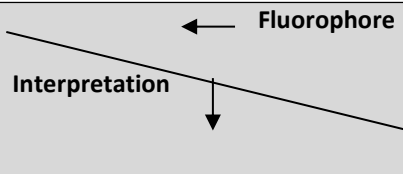
***Plate read/Data acquisition in FAM and Yakima Yellow/ VIC/ HEX Channel**

Sample analysis and Interpretation

The criteria for the acceptance of the assay should be met before the interpretation of the results. The JAK-2 PC amplification signal is only detected in the FAM channel. JAK-2 NC amplification signal is only detected in Yakima Yellow/VIC/HEX channel. No amplification should be observed in either of the channels for NTC.

| Controls | FAM (JAK-2) | Yakima Yellow / VIC/HEX (Endogenous control) |
|----------|----------------|---|
| JAK2 PC | √ | - |
| JAK2 NC | - | √ |
| NTC | - | - |

Interpret the values for unknown samples based on the observations described in the following table. The mutation are detected in the FAM channel, while the internal control is detected in Yakima Yellow/VIC/HEX channel. The Ct values of ≤ 40 Ct for JAK-2 DNA and ≤ 30 Ct for endogenous control of Unknown samples should be considered for positive sample interpretation.

| S. No | FAM (JAK-2) | Yakima Yellow / VIC/ HEX (Endogenous Control) |  | Conclusion |
|-------|----------------|--|--|---|
| 1 | √ | √ | Mutation Detected | Proceed for further Analysis |
| 2 | - | √ | Mutation Not Detected | |
| 3 | - | - | Possible inhibition of PCR | Make sure reaction setup and PCR conditions are followed correctly Recheck quality and quantity of DNA |

Note: All the Target channels (FAM, Yakima Yellow/VIC/HEX) to be analyzed individually.

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96



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Quality management system is certified in compliance with the requirements of ISO 9001:2015 and ISO 13485:2016