JAK2 Real-time PCR Kit

(For Qualitative Detection)

REF 8501

INTENDED USE

JAK2 Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of JAK2 1849 (G→T) V617F mutation allele in genomic DNA extracted from EDTA whole blood.

KIT CONTENTS

Components	Number of vials	Volume Per vials
Probe PCR Master Mix	1	250µl
JAK2 Primer Probe Mix [JAK2 PP mix]	1	65µl
Endogenous Primer Probe Mix [Endogenous PP Mix]	1	65µl
JAK2 Positive control	1	150µl
Water, PCR grade	1	4ml

STORAGE

1. The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

PRODUCT DESCRIPTION

JAK2 V617F mutation Real-time PCR Kit constitutes a ready-to-use system for the detection of the point mutation 1849 (G→T) V617F in the JAK2 gene occurs at high frequency in several chronic myeloproliferative diseases using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification for the direct detection of the specific amplicon in fluorescence channels FAM. In addition, it contains an endogenous control amplification system amplifies human gene detected in HEX channel which identify possible PCR inhibition and DNA purification. External mutation positive control is supplied to assist the run.

SPECIFICITY

JAK2 mutation primer and probe have been designed for the specific and exclusive in vitro detection of 1849 (G→T), in the JAK2 human gene [GenBank accession no. NM_004972]. The primers and probe sequences in this kit have 100% homology with clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of DNA molecules (ng/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified JAK2 specific DNA from 1ng/µl to 100ng/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 2ng/µl

DNA PURIFICATION

Strongly recommended to use minimum 300µl of EDTA whole human blood for the DNA purification.

If you are using a spin column-based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

ENDOGENOUS CONTROL

Human gene is given as endogenous control. It amplifies a single copy human gene from the test samples. A successful amplification indicates that test samples are properly collected and has its biological property.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the endogenous control template does not interfere with detection of the mutation even when present at low copy number. The endogenous control is detected through the HEX channel and gives a CT value of 23 +/-7.

DETECTION PROTOCOL

1. Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
2. Make sure that Positive and Negative control is included in every run.
3. Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions

Components	Volume per reaction
Probe PCR Master Mix	10µl
JAK2 PP Mix	2.5µl
Endogenous PP Mix	2.5µl
Master Mix Volume	**15µl**
Purified DNA	**10µl**
Final reaction volume	25µl

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Positive Control setup

Add 10µl of the Positive control

PROGRAMMING THERMAL CYCLER

Sample volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference	None
Ramping rate	Default

THERMAL PROFILE

Cycles	Step	Time	Temp
1	Taq enzyme activation / Hold	15min	95ºC
45	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	56ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	JAK2
HEX	Endogenous control [Human gene]

READING THE GRAPH

Step-1 –Endogenous control Validation

Select the test samples alone for the endogenous control analysis. Select HEX dye and view the graph of endogenous control amplification. A successful amplification Ct value must be within Ct 25 +/- 7.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 32 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

Step-2 – FAM - Negative and Positive control validation

Select the NTC and Positive control, select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The Positive control must be amplified.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-3 –FAM - Test Sample status

In FAM channel, select test sample well one by one, analyze the graph/amplification.

QUALITATIVE INTERPRETATION OF RESULTS

Test Sample	Negative Control	Positive Control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	JAK2 mutation specific DNA detected
Negative	Negative	Positive	Positive	Not Detected
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

LIMITATIONS

A false negative result may occur due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the JAK2 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

In accordance with in house Quality Management System, each lot of JAK2 Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Index of symbols

Consult instructions for use	LOT Batch code	Tests per kit
IVD For *in vitro* diagnostic use only	Do not re-use	Keep dry
Temperature limit - 25 °C to - 15 °C	Date of manufacture	Biologial Risks
Manufacturer	Use-by date	

Marketed by:

Athenese-Dx Pvt. Ltd.

PI-8501 Rev. A
Effective date: 05.08.2020
English version