SinaClon
شرکت سیناکلون BioScience

JAK2 V617F Detection Kit

For Real-Time PCR

REF **PQ3641** | Σ **25 TESTS** | RUO

Wet or dry Ice | **Store at: -20°C**

Components

Contents	No. of Tubes	Vol. per Tube (µl)
2X Master Mix Probe	1	250µl
JAK2-V617F Detection Primer/Probe Mix*	1	175µl
Control Positive 1% JAK2 V617F mutant	1	20µl

*The primer mix tube contains primers/probes for both JAK2 V617F (FAM) and endogenous control gene (HEX).

For Use With:

- Applied Biosystems® - 7500/7500 Fast, StepOne/ StepOne Plus
- Qiagen Rotor-Gene® Q
- LightCycler® 480
- QuantStudio™ 5/6
- Applied Biosystems® - 7900HT
- Qiagen Rotor-Gene® 3000, 6000
- Cobas® 4800, Cobas® z480
- Bio-Rad® CFX96

Intended use

The chronic myeloproliferative Philadelphia-negative diseases (CMPD) are a group of stems hemopoietic disorders including the polycythemia vera (PV), the essential thrombocytosis (TE) and the idiopathic myelofibrosis (CIMF). In 2005 the presence of a somatic mutation (V617F) of gene Janus Kinase 2 (JAK2), coding for an important protein for signal transduction inducted by hemopoietic growth factors, in patients CMPD Ph negative has been shown; it phosphorylates different cytoplasmic molecules, particularly the STAT (Signal Transducers and Activators of Transcription). The protein JAK2 belongs to the Janus kinases family; these proteins are composed by seven regions: JH1-JH7. Particularly, JH1 is the kinase action region. JH2 is pseudo-kinase domain, important for JH1 catalytic activity and involved in the inhibitory regulation of that activity. The mutation V617F, is caused by the nucleotide substitution G>T at nucleotide 1849 in the exon 14, inducing the substitution of the valine amino acid with phenylalanine at codon 617 (GTC>TTC). This mutation involves a portion of the pseudo-kinase JH2 of JAK2, important in controlling JH1 inhibitory activity. This is an acquired somatic mutation, that can be found only in myeloid cells (erythroid line, granulocyte-macrophage line, megakaryocyte line) in the heterozygous and homozygous state. The mutation is called “gain-of-function mutation” because it determines a constituent activation of the JAK-STAT pathway, able to give a proliferative advantage and cytokine-independent growth of the hemopoietic cells.

The mutation V617F has been often noticed in patients with:

- Polycythemia vera (65-97%), 20% more than other homozygous patients.
- Essential thrombocytosis (23-57%), generally present in heterozygous form.
- Myelofibrosis (35-95%)

Less frequently V617F has been found in other diseases such as chronic myelomonocytic leukemia, the myelodysplastic diseases (MDS), systemic Masto cytosis (SM), the chronic neutrophilic leukemia, the hypereosinophilic syndrome and the atypical chronic myeloproliferative diseases. The mutation JAK2- V617F has never been found in healthy subjects. The mutation’s study can explain the disease and can be used as a diagnostic and prognostic.

Reagents

Reagent preparation (for all instruments)

The reactions are setup in a total volume of 20 µl. Reaction mixes for multiple samples (as well as control samples) should be pre-mixed as a master mix with 5% excess volume to compensate for pipetting losses.

Each 25µl reaction contains the following components:

Component	Volume (µl)
2x Master Mix Probe	10
JAK2 V617F Detection Primer/ Probe Mix	7
DNA sample	3

Result Interpretation

Evaluation of Extracted DNA quality and Real-time PCR procedure Endogenous Control Gene:

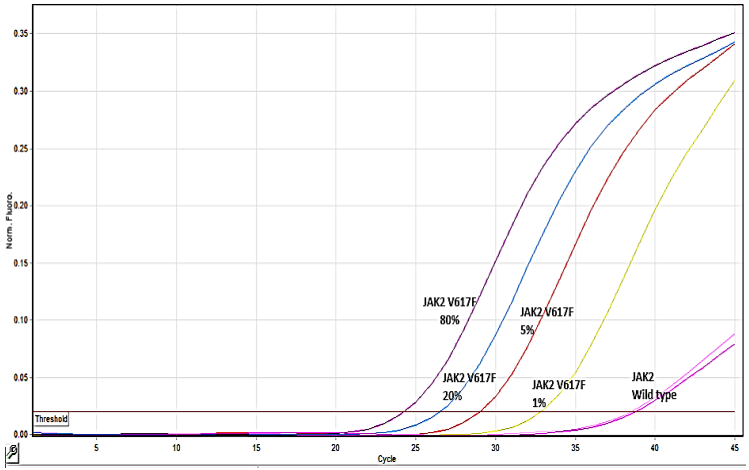
Endogenous control Gene Ct is:	Control Status
24 ≤ Ct ≤ 30	Sufficient DNA
24 > Ct	Overloaded reaction. May observe non-specific signal. Re-test sample with less DNA to bring HEX within acceptable range.
Ct >30	Low DNA concentration. A rerun with more DNA is recommended.

Interpretation of Target Gene JAK2 V617F

ΔCt: Ct (FAM) Target Gene JAK2 V617F – Ct (HEX) Endogenous Control Gene	Mutation Status
ΔCt > 6	Negative
2 < ΔCt ≤ 6	Heterozygote Positive
ΔCt ≤ 2	Homozygote Positive

Limit of Detection

The reaction is setup for detection of 1% mutation JAK2 V617F



Signs

Signs	Definitions	Signs	Definitions
RUO	For Research Use Only		Product shipping conditions
	Name and address of the manufacturer of the product	REF	Product technical code

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