JAK-2 QUANTITATIVE REAL TIME PCR KIT
Cat. No: 21QR-10-01

PRODUCT DESCRIPTION

The *JAK2* V617F mutation is an acquired, somatic mutation present in the majority of patients with myeloproliferative cancer (myeloproliferative neoplasms) i.e. nearly 100% of patients with polycythemia vera and in about 50% of patients with essential thrombocytosis and primary myelofibrosis.

PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5′ end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Each isolated DNA should be tested with wild type and mutant type real time pcr mastermixes. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5′ nuclease PCR. The test system is designed by SNP for use with sequence specific primers and probe.
The fluorescence dye used for mutation analysis is FAM. Also each mastermix contains an internal control labelled with HEX/JOE dye.

SYSTEM CONTENTS

Reagents	20 rxns
• JAK-2 Wild-Type PCR mastermix	400 µl
• JAK-2 Mutant PCR mastermix	400 µl
• Quantification PCR mastermix	1200 µl
• Control DNA	30 µl
• Quantification Standards (QS)*	50 µl

* JAK-2 Quantitative Real-Time PCR Kit contains Quantification Standards (QS) at six different concentrations (Concentration of quantification standards QS1, QS2, QS3, QS4, QS5 and QS6 are respectively $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$, $1x10^2$ copy/5 µl).

DNA EXTRACTION

Blood samples should be collected in appropriate sterile EDTA tubes and can be stored at +4°C up to one month. For more than one month specimen should be stored at -20°C. It is advised to gently mix the tube (with EDTA) after collection of blood to avoid coagulation.
Our system is optimized according to MN NucleoSpin ® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

PROCEDURE

- Different wild- type and mutant tubes should be prepared.
- Before starting work, mix the mastermixes gently by pipetting.
- For each sample, pipet **20 µl mastermix*** with micropipets of sterile tips to each optical white strips or tubes.
- Add **5 µl (~1-100 ng) DNA** into each tube**.**
- Place and run together with the quantification standards according to the programme shown below.

****Master mixes include HotStart Taq DNA Polymerase.***

For quantification standards;

- In order to make quantification standard curve, pipet **20 µl of Quantification PCR Mastermix** to six different optical white strips or tubes.
- Add **5 µl of QS1, QS2, QS3, QS4, QS5 and QS6** respectively into six different tube**.**
- Place and run together with the samples according to the programme shown below.
- Select well type as **"Standard"** and enter **QS values*** of quantification standards in order to calculate standard curve. The standard curve will be automatically calculated by the equipment software.

****Please check the values in "system contents" part.***

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	32 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM and HEX/JOE.

This system can use with;

ABI Prism ® 7500/7500 Fast
Bio-Rad CFX96
Rotor Gene Q
Roche LightCycler® 480

If you use;

- ABI Prism® system, please choose **"none"** as passive reference and quencher.

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DATA ANALYSIS

After the run is completed, data are analysed using the software with HEX (JOE) and FAM dyes. The below results were studied with BioRad CFX96.

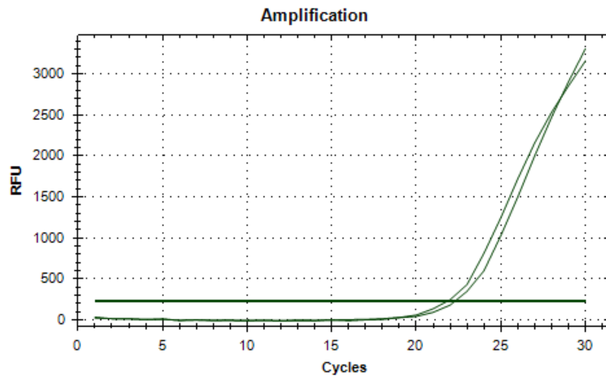

Figure 1: Internal Control plots – HEX/JOE Dye

Internal control amplification plots, labelled with HEX/JOE dye must be seen in all wells except NTC. The CT value of internal controls should be **20 ≤ X ≤ 26**.

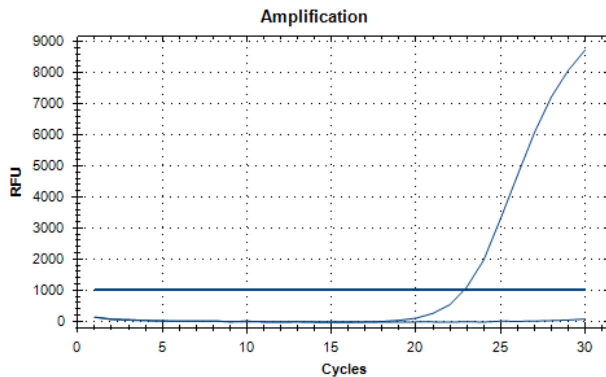

Figure 2: Wild type sample (only amplified with wild type mix) – FAM Dye

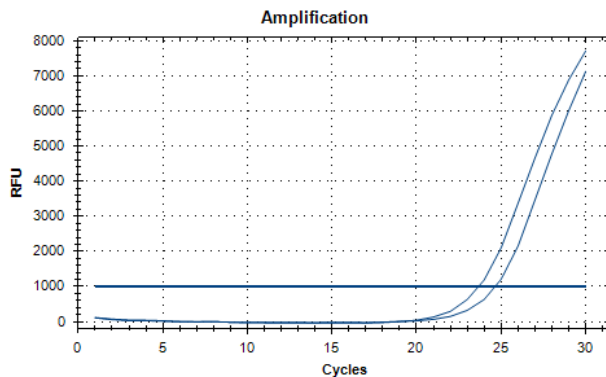

Figure 3: JAK-2 positive sample
(amplified both with wild type and mutant mix) – FAM Dye

Amplification plots of JAK-2 mutation can be analysed by FAM dye. The CT value should be between **20 ≤ CT ≤ 29**. These values are optimised according to the SNPure® Blood DNA Isolation Kıt and MN NucleoSpin ® Blood DNA Isolation Kıt. CT values may vary ±2/3 cycle according to the DNA isolation kits.

The limit of detection (LOD) in Jak-2 Real Time PCR Kit was determined as ≤ 1% Jak-2 mutation.

Quantification of JAK-2

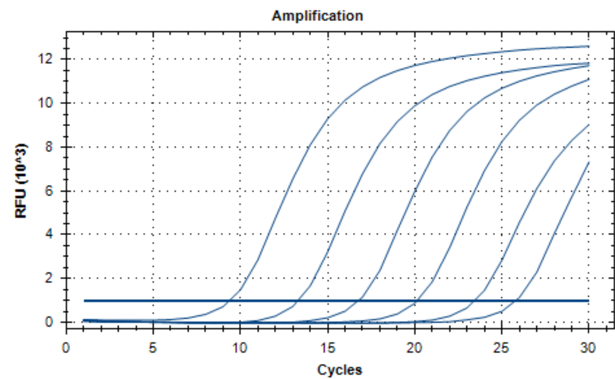

Figure 4: Amplification plots of quantification standars – FAM Dye

Step 1

Threshold baseline value of FAM dye should be adjusted to 1000 (for BioRad CFX96) to calculate appropriate mutation percentage.

Please contact with tech@snp.com.tr for required threshold settings of other equpments.

Step 2

Well type of quantification standards should be selected as **"Standard"** and add **value of QS** needs to be added in order to perform standard curve. After these steps the standard curve will be automatically calculated by the equipment software (Figure 5).

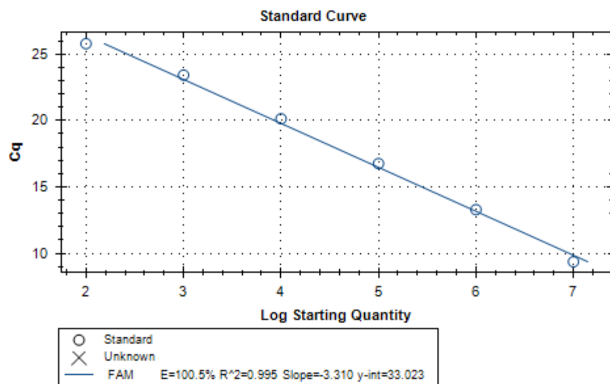

Figure 5: Standard curve – FAM Dye

Ideal slope value for standard curve should be -3≥ slope ≥ -3,6. Below and above any slope value may affect the result of mutation percentage.

Step 3

Following Step 1 and Step 2, real time PCR software will automatically calculates the SQ (Standard Quantification) values for each well.

Step 4

By using the equation below, the percentage of the mutation can be easily calculated;

Mutation Percetage (%) = SQ Mutant/ (SQ Wild type + SQ Mutant)

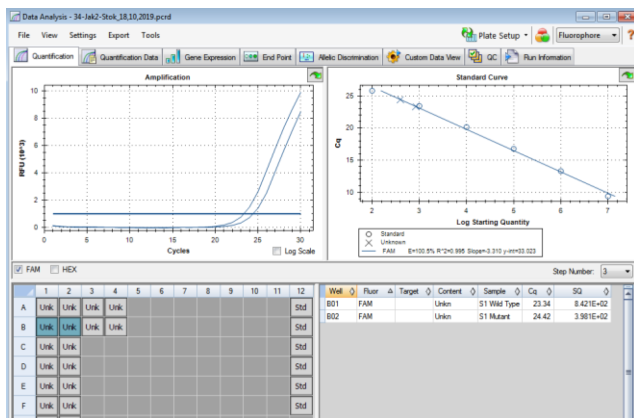

Figure 6: Result of a JAK-2 positive sample

Well	Fluor	Target	Content	Sample	Cq	SQ
B01	FAM		Unkn	S1 Wild Type	23.34	8.421E+02
B02	FAM		Unkn	S1 Mutant	24.42	3.981E+02

Figure 7: SQ values of JAK-2 positive sample

Mutation percentage for the example study can be calculated as;

$\% = 3,98 \times 10^2 / (8,42 \times 10^2 + 3,98 \times 10^2)$

From the equation it can be found as S1 sample is carrying 32% of JAK-2 mutation.

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- DNA quality is not good.
- The amount of DNA is not enough.

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>3X) should be avoided, as this may reduce the sensitivity of the assay.