**SNP Biotechnology R&D Ltd.**
Hacettepe Technopolis 1. R&D Building Office No: 10 Beytepe – ANKARA / TURKEY
Tel : +90312-2992328 / Fax : +90312-2992348 / www.snp.com.tr / info@snp.com.tr

# JAK2 QUANTITATIVE REAL-TIME PCR KIT
# Cat. No: 21QR-10-01

## INTRODUCTION

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[1].

## INTENDED USE

JAK2 Quantitative Real-Time PCR Kit is designed to detect the V617F mutation of the JAK2 gene in whole blood samples by using quantitative Real-Time PCR method.

## TARGETED USER

For professional use only. Testing should be performed by professionals trained in molecular techniques.

## 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 ($C_T$) is proportional to the amount of the specific PCR product[2,3].

## PRODUCT SPECIFICATION

Each isolated DNA should be tested with wild type and mutant master mixes. The kit provides reagents in a **"ready-to-use"** master mix format which has been specifically adapted to 5′ nuclease PCR for SNP analysis. The test system is designed by SNP Biotechnology for use with sequence specific primers and probes.
The fluorescence of mutation analysis is FAM. Also each master mix contains an internal control labelled with HEX/JOE dye. Internal Control is Prothrombin gene – FII (OMIM: 176930).

## SYSTEM CONTENTS

| Reagents | 10 rxns | 20 rxns | 50 rxns |
|---|---|---|---|
| JAK2 Wild Type PCR Master Mix | 200 µl | 400 µl | 1000 µl |
| JAK2 Mutant PCR Master Mix | 200 µl | 400 µl | 1000 µl |
| Quantification PCR Master Mix | 600 µl | 1200 µl | 2400 µl |
| Quantification Standards (QS1 - 1x10$^6$)* | 50 µl | 50 µl | 100 µl |
| Quantification Standards (QS2 - 1x10$^5$)* | 50 µl | 50 µl | 100 µl |
| Quantification Standards (QS3 - 1x10$^4$)* | 50 µl | 50 µl | 100 µl |
| Quantification Standards (QS4 - 1x10$^3$)* | 50 µl | 50 µl | 100 µl |
| Control DNA** | 30 µl | 30 µl | 60 µl |

**Table 1:** Kit content

* JAK2 Quantitative Real-Time PCR Kit contains Quantification Standards (QS) at four different concentrations (Concentration of quantification standards QS1, QS2, QS3 and QS4 are respectively 1x10$^6$, 1x10$^5$, 1x10$^4$, 1x10$^3$ copy/5 µl). Please gently vortex and then spin centrifuge for 1-2 seconds before use the positive control.
** Control DNA is a synthetic plasmid containing the mutation regions. Expected results for synthetic control DNA should be JAK2 V617F positive. Since to Control DNA is a synthetic plasmid, amplification plots of synthetic control DNA may appear slightly different from the sample DNA. Please gently vortex and then spin centrifuge for 1-2 seconds before use the positive control.

## 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 ( >4X) should be avoided, as this may reduce the sensitivity of the assay.

## SAMPLE COLLECTION

JAK2 Quantitative Real-Time PCR Kit is approved for use with whole blood samples.
- Standard precautionary instructions must be followed by all healthcare professionals during the collection and transportation of whole blood samples.
- Whole blood samples should be collected in appropriate containers before delivery to the laboratory.
- Freezing and thawing of samples should be avoided.

## 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 optimized according to GeneAll® Exgene™ Blood SV. It is advised to elute DNA with 150 µl elution buffer for better results.

Before SNP Kits using, DNA concentration measurement and adjustment **should not be** performed.

## PROCEDURE

- Different test tubes should be prepared for each master mix.
- Leave the master mixes* and controls at RT to melt.
- Before starting work, mix the master mixes gently by pipetting
- For each sample, pipet **20 µl master mix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube. Please do not pipette DNA before and after addition into well.
- Optical caps are closed, it is recommended to spin the plates/strips at low speed for a short time.
- Run with 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 Master mix** to four different optical white strips or tubes.
Add **5 µl of QS1, QS2, QS3, QS4** respectively into four 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 (Figure 7).
***Please check the values in "system contents" part.***

## PCR PROGRAMME

| | | |
|---|---|---|
| 96 °C | 1 Min. | Holding |
| 96 °C | 5 Sec. | 32 Cycles |
| 60 °C | 45 Sec. | |

**Table 2:** PCR Programme

Fluorescent dyes are FAM and HEX/JOE.
**This system can be used with the following devices;**
- Bio-Rad CFX96
- ABI Prism® 7500/7500 Fast
- Roche LightCycler® 480 System
- Rotor Gene Q
- Mic qPCR Cycler

For other two or more channel Real-Time PCR devices (which can read FAM and HEX/JOE dyes), a trial run is recommended.

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**If you use;**
ABI Prism® system, please choose **"none"** as passive reference and quencher.
Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10**.

**Supplied Materials**
- White PCR plates/strips with optical covers*

*The PCR Plate/strip tube and caps seriously affect the amplification curve quality. Therefore, white PCR plates/strips and optical caps provided by the manufacturer should be used with the kit.

**Required Materials (Not Provided)**
- PCR Cabinet
- Vortex Mixer
- Desktop Microcentrifuge (For 2.0ml tubes and PCR strip tubes), plate spin for studies using PCR plates.
- Automated or spin column based DNA isolation Kit
- Disposible powder-free laboratory gloves
- Micropipettes (0.5ml-1000ml)
- Micropipette tips
- Standard laboratory equipments.

## 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.

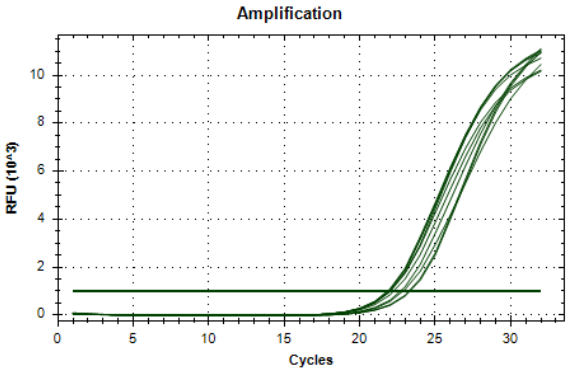


**Figure 1:** Internal Control plots – HEX/JOE Dye

Internal control amplification plots, labelled with HEX/JOE dye must be seen in all wells except NTC. The $C_T$ value of internal controls should be **20 ≤ X ≤ 28**

Amplification plots of JAK2 mutation can be analysed by FAM dye.
**The limit of detection (LOD) in JAK2 Real Time PCR Kit was determined as ≥1% JAK2 mutation.**
**Performance of JAK2 Quantitative Real Time PCR Kit is verified by 100% compatibility using internationally accepted NIBSC Reference controls, NIBSC/ WHO 1st International Reference Panel for Genomic JAK2 V617F - 16/120** [4].

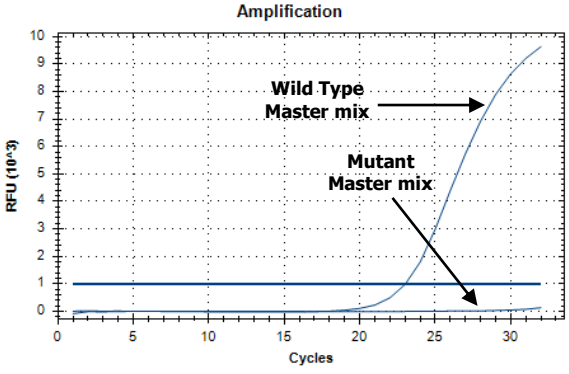


**Figure 2:** Wild type sample (only amplified with wild type mix) – FAM Dye

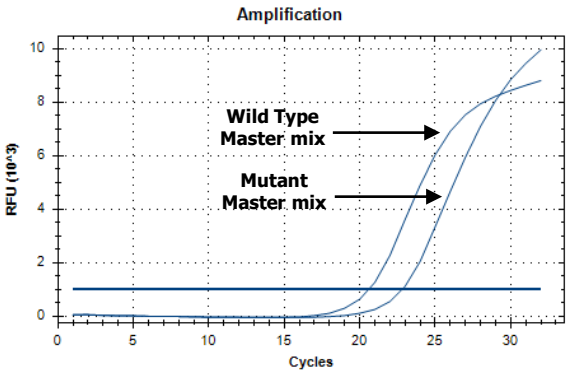


**Figure 3:** JAK2 positive sample (amplified both with wild type and mutant mix) – FAM Dye

### Quantification of JAK2

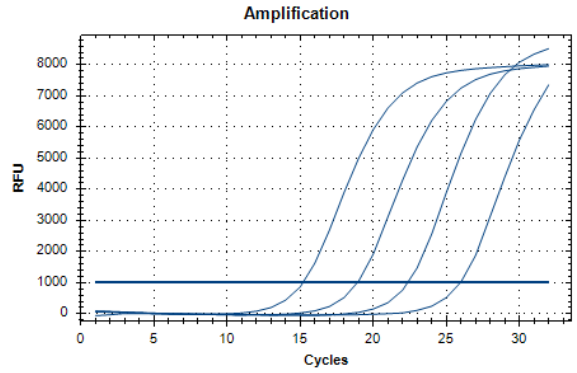


**Figure 4:** Amplification plots of quantification standars – FAM Dye

### Step 1

Threshold baseline value of FAM dye should be adjusted to 300-1000* (for BioRad CFX96) to calculate appropriate mutation percentage.
Please contact with tech@snp.com.tr for required threshold settings of other equipments.
*Depending on the DNA isolation system, the threshold value on CFX96 instruments can be adjusted within this range.

### 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).

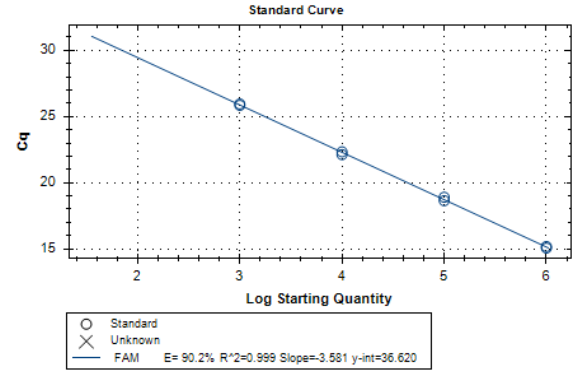


**Figure 5:** Standard curve – FAM Dye

Ideal slope value for standard curve should be -3,1 ≥ slope ≥ -3,8. Below and above any slope value may affect the result of mutation percentage.

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### 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 Percentage (%) = SQ Mutant/ (SQ Wild type + SQ Mutant)

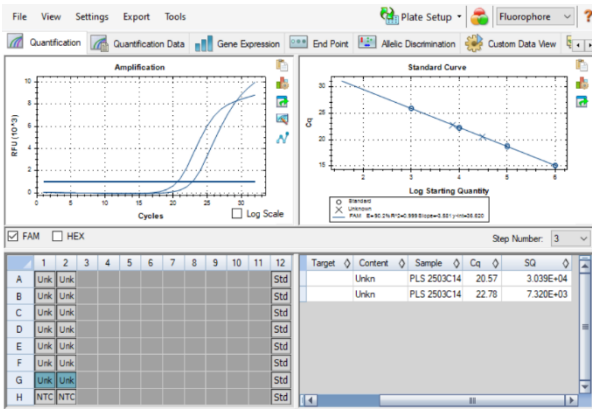


**Figure 6:** Result of a JAK2 positive sample

| Well | Fluor | Target | Content | Sample | Cq | SQ |
|---|---|---|---|---|---|---|
| G01 | FAM | | Unkn | PLS 2503C14 | 20.57 | 3.039E+04 |
| G02 | FAM | | Unkn | PLS 2503C14 | 22.78 | 7.320E+03 |

**Figure 7:** SQ values of JAK2 positive sample

Mutation percentage for the example study can be calculated as;

Mutation Percentage (%) = $7.320 \times 10^3$ / ($3.039 \times 10^4$ + $7.320 \times 10^3$)

From the equation it can be found as sample is carrying 19% of JAK2 mutation.

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

## DISPOSAL OF KIT
Dispose of it according to the legal regulations of your region

## SYMBOLS AND DESCRIPTIONS

| | | | |
|---|---|---|---|
| REF | Catalog Number | CE | CE Mark |
| LOT | Lot Number | UDI | Unique Device Identifier<br>(01)Device Identifier<br>(17)Expiry Date<br>(10)Lot Number |
| | Manufacturer | Σ | Test Quantity |
| | Fragile | -20 °C | Storage Temperature |
| | Protect from directly sunlight | IVD | In Vitro Diagnostics |
| | Expiry Date | | |

**Table 3:** Symbols and descriptions

## REFERENCES
1. Alexandra P. Wolanskyj, Terra L. Lasho, Susan M. Schwager, Rebecca F. McClure, Martha Wadleigh, Stephanie J. Lee, D. Gary Gilliland and Ayalew Tefferi. "JAK2V617F mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance". British Journal of Haematology,2005, 131, 208–213.
2. Yolanda S Lie and Christos J Petropoulos. "Advances in quantitative PCR technology: 5' nuclease assays". Current Opinion in Biotechnology Volume 9, Issue 1, February 1998, Pages 43-48.
3. Luis Ugozzoli and R. Bruce Wallace. "Allele-Specific Polymerase Chain Reaction". A Companion to Methods in Enzymology Vol. 2, No. 1, February, pp. 42-48, 1991.
4. WHO Reference Panel 1st International Reference Panel for Genomic JAK2 V617F NIBSC code: 16/120 Instructions for use (Version 4.0, Dated 25/04/2020).

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## TROUBLESHOOTING PROBLEMS AND SOLUTIONS

| Problem | Reason | Solution |
|---|---|---|
| Internal control does not work/ low amplification | Absence of DNA / DNA extraction problems | Repeat test |
| | Absence of DNA / DNA extraction problems | • DNA extraction should be repeated.<br>• DNA extraction should be replaced with one of the recommended methods. |
| | Sample is containing PCR inhibitor(s) | |
| No target gene amplification curves in some samples for both wild type and mutant mixes. | Absence of DNA / not added into well | Repeat test |
| | Absence of DNA / DNA extraction problems | • DNA extraction should be repeated.<br>• DNA extraction should be replaced with one of the recommended methods. |
| | Sample is containing PCR inhibitor(s) | |
| No target DNA/internal control amplification curves in all wells | Error in temperature/time settings in PCR program | Correct any errors in the temperature/time settings in the PCR Program and repeat the test. |
| | Sample is containing PCR inhibitor(s) | • DNA extraction should be repeated.<br>• DNA extraction should be replaced with one of the recommended methods. |
| Positive control result and/or $C_T$ values are lower or higher than the value mentioned in User Manual. | Error in temperature/time settings in PCR program | Correct any errors in the temperature/time settings in the PCR Program and repeat the test. |
| $C_T$ values are not valid (higher or lower) according to User Manual | Excessive or insufficient DNA sample | • Repeat the test.<br>• DNA extraction should be repeated. |
| Low and/or invalid amplification curves | Stability problems arising from repeated thawing and freezing ( >4X) | Repeated thawing and freezing ( >4X) should be avoided, as this may reduce the sensitivity of the assay. |
| | Sample is containing PCR inhibitor(s) | • DNA extraction should be repeated.<br>• DNA extraction should be replaced with one of the recommended methods. |
| | Stability problems arising from unavailable storage conditions. | All reagents should be stored at – 20 °C and dark. |
| | Bubble formation or pipetting error during pipetting | After adding the master mix and sample, it is recommended to spin the plates/strips at low speed for a short time. |
| For further questions, please contact us **tech@snp.com.tr** | | |

**Table 4:** Troubleshooting problems and solutions