

JAK2 EXON 12 REAL-TIME PCR KIT
Cat. No: 23R-10-04

INTRODUCTION

In the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues, Philadelphia-negative myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Somatic mutations of JAK2 exon 12 are found in the remaining 5% of patients with PV (1).

INTENDED USE

JAK2 Exon 12 Real-Time PCR Kit screens JAK2 gene Exon 12 mutations on table 5 in whole blood samples by using qualitative 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 with the amount of **mutant** PCR products. System gives amplification plots only if there is any mutation in the sample DNA except internal control. Wild type sample amplifies only with internal control.

In order to analyze some regions, wild type sequences are blocked by specifically designed oligonucleotides. It gives perfect opportunity to screen mutations, since there is not any prevention in mutant types (2,3).

PRODUCT SPECIFICATION

Each isolated DNA should be tested with all mixes separately. 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 dyes used for mutation analysis are FAM. Also each master mix contains an internal control labelled with HEX/JOE dye. Internal Control is Prothrombin gene – FII (OMIM: 176930). Mutations and related dyes can be seen in Table 3.

SYSTEM CONTENTS

Reagents	10 rxns	20 rxns	50 rxns
Exon 12 Mix 1	200 µl	400 µl	1000 µl
Exon 12 Mix 2	200 µl	400 µl	1000 µl
Exon 12 Mix 3	200 µl	400 µl	1000 µl
Exon 12 Mix 4	200 µl	400 µl	1000 µl
Control DNA*	75 µl	75 µl	150 µl

Table 1: Kit content

*Since to control DNA is a synthetic plasmid, amplification plots of synthetic control DNA may appear slightly different from the sample DNA. Amplifications of control DNA can be found in Table 2. Please gently vortex and then spin centrifuge for 1-2 seconds before use the Control DNA.

Exon 12 Control DNA				
MIX 1	MIX 2	MIX 3	MIX 4	DYES
+	+	+	+	HEX/JOE
+	+	+	-	FAM

Table 2: Amplifications of Exon 12 Control DNA

MUTATION / DYE TABLE

Tubes	Mutations	Dyes
Mix 1	JAK-2 exon 12	FAM
	Internal Control	HEX/JOE
Mix 2	JAK-2 exon 12	FAM
	Internal Control	HEX/JOE
Mix 3	JAK-2 exon 12	FAM
	Internal Control	HEX/JOE
Mix 4	JAK-2 exon 12	FAM
	Internal Control	HEX/JOE

Table 3: Tubes- mutations- dyes.

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 Exon 12 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.

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.

PCR PROGRAMME

96 °C	1 Min.	Holding
96 °C	2 Sec.	
60 °C	30 Sec.	

32 Cycles

Table 4: PCR Programme

Fluorescent dyes are FAM and HEX/JOE.

This system can be used with the following devices;

- Bio-Rad CFX96, Opus 96
- 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), a trial run is recommended.

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
- Disposable 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 FAM, and HEX/JOE dyes. The below results were studied with Bio-Rad CFX96. The threshold values for all dyes were set to 1000, based on experiments conducted using the Bio-Rad CFX96 Real-Time PCR system, the GeneAll® Exgene™ Blood SV Isolation Kit, and white PCR strips supplied by SNP Biotechnology. Threshold values may vary depending on the PCR device, DNA isolation kit, and the type or brand of PCR strips/tubes used.

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The C_T value of internal controls should be 20 ≤ C_T ≤ 28. These values are optimised according to the GeneAll® Exgene™ Blood SV Isolation Kit and Bio-Rad CFX96 Real-Time PCR Device. C_T values may vary ±2/3 cycle according to the other DNA isolation systems and Real-Time PCR devices.

Amplification plots of JAK2 Exon 12 mutations can be analysed by FAM dye. For sample which has acceptable internal control range, If the these dyes signal in the mixes exceeds the threshold level sample evaluate as positive for mutation (Table 3 and 5).

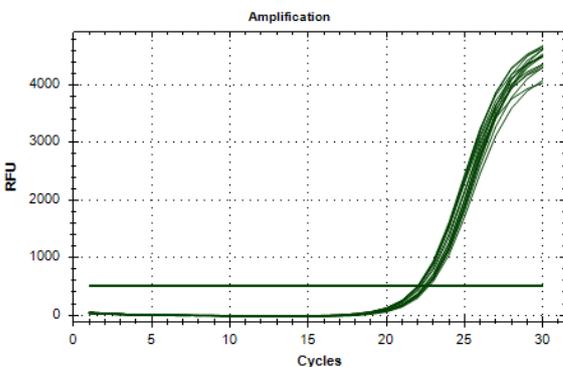


Figure 1: Internal Control plots – HEX / JOE Dye

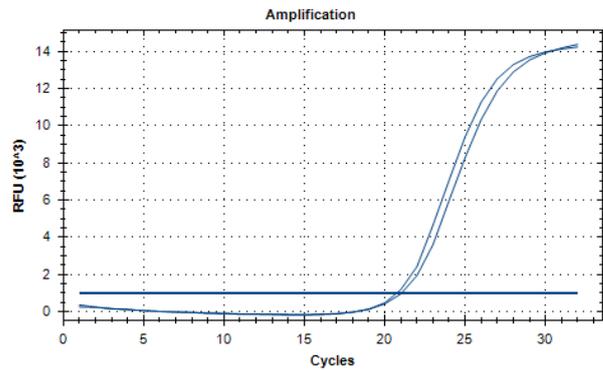


Figure 2: JAK-2 Exon 12 Positive Result- FAM dye - Mix1 and Mix3

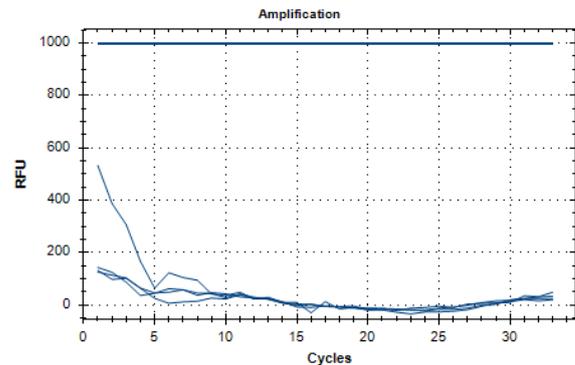


Figure 3: JAK-2 Exon 12 Negative Result- FAM dye

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR master mixes forgotten at room temperature.
- Thaw PCR master mixes 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.
- Can be used for Research Use Only.

DISPOSAL OF KIT

Dispose of it according to the legal regulations of your region

REFERENCES

1. Rumi E, Pietra D, Ferretti V, Klampfl T, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood*. 2014; 123(10):1544-1551
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)

Mutations List for Jak2 Exon12

Mix 1 (FAM)	F537IK539L, K539L, F533IK539L, H538QK539L, H538DK539LI540S, K539LL545V, H538del, H538-K539delinsL, H538-K539delinsI, H538-K539delinsF, H538-K539del
Mix 4 (FAM)	V536-I546dup11, V536-F547dup12, (V536F,F37-I546dup10), F537-F547dup11, (F537-I546dup10, F547L), (F547L, I540-F547dup8)
Mix 1 and/or Mix 2 (FAM)	F537-K539delinsK, F537-K539delinsL, F537-K539del
Mix 1 and/or Mix 3 (FAM)	I540-N542delinsS, I540-N542delinsK, I540-E543delinsKK, N542-E543del, R541-E543delinsK, (I540S, R541-E543delinsK), I540-E543delinsMK, I540-D544delinsMK, E543-D544del, N542-D544delinsN, R541-D544del, D544-L545del

Table 5: List of mutations screened by dyes and mixes

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	<ul style="list-style-type: none"> DNA extraction should be repeated. 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)	<ul style="list-style-type: none"> DNA extraction should be repeated. 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	<ul style="list-style-type: none"> Repeat the test. 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)	<ul style="list-style-type: none"> DNA extraction should be repeated. 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 6: Troubleshooting problems and solutions

SYMBOLS AND DESCRIPTIONS

	Catalog Number		Research Use Only
	Lot Number		Test Quantity
	Manufacturer	 -20 °C	Storage Temperature
	Fragile		
	Protect from directly sunlight		
	Expiry Date		

Table 7: Symbols and descriptions