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MPN SCREENING KIT
Cat. No: 23R-20-10

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).
In recent studies it was found that, three-quarters of patients carry the unique JAK2 (V617F) mutation, which is present in about 95% of subjects with PV and in about 60% of those with ET or PMF. Somatic mutations of JAK2 exon 12 are found in the remaining 5% of patients with PV, whereas mutations of MPL exon 10 are present in about 5% of those with ET or PMF. Most patients with ET or PMF with nonmutated JAK2 and MPL carry a somatic mutation of CALR, the gene encoding Calreticulin[1].

INTENDED USE
MPN Screening Kit screens JAK-2 gene Exon 12 mutations; between amino acids 530 and 547, JAK-2 Exon 14 gene V617F mutation, MPL gene Exon 10; S505N, W515A, W515K, W515RL, W515R mutations, CALR gene Exon 9; Type 1, Type 2 and other mutations and CSF3R gene; T615A and T618I mutations.
Please check the mutation lists at the table 3 and table 5 for detailed information about the mutations that can be screened with the kit.

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, HEX/JOE and Texas Red. Also each master mix contains an internal control labelled with CY5 dye. Internal Control is Prothrombin gene – FII (OMIM: 176930). Mutations and related dyes can be seen in Table 3.
The kit offers sensitivity to detect under 1% mutant allele in background of 99% wild type allele in the related mutations [4] .

SYSTEM CONTENTS

Reagents	10 rxns	20 rxns	50 rxns
MPN Mix 1	200 µl	400 µl	1000 µl
MPN Mix 2	200 µl	400 µl	1000 µl
MPN Mix 3	200 µl	400 µl	1000 µl
MPN 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.

MPN Control DNA				
MIX 1	MIX 2	MIX 3	MIX 4	DYES
+	+	+	+	CY5
+	+	+	-	FAM
+	+	-	+	HEX/JOE
+	-	+	+	Texas Red

Table 2: Amplifications of MPN Control DNA

MUTATION / DYE TABLE

Tubes	Mutations	Dyes
Mix 1	JAK-2 exon 12	FAM
	CALR	Texas Red
	T615A	HEX/JOE
	Internal Control	CY5
Mix 2	JAK-2 exon 12	FAM
	CALR	Texas Red
	T618I	HEX/JOE
	Internal Control	CY5
Mix 3	JAK-2 exon 12	FAM
	CALR	Texas Red
	S505N & W515L & W515R	HEX/JOE
	Internal Control	CY5
Mix 4	JAK-2 exon 12	FAM
	JAK-2 V617F	Texas Red
	W515A & W515K	HEX/JOE
	Internal Control	CY5

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
MPN Screening 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.

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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.	32 Cycles
60 °C	30 Sec.	

Table 4: PCR Programme

Fluorescent dyes are FAM, HEX/JOE, Texas Red and CY5.

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 four or more channel Real-Time PCR devices (which can read FAM, HEX/JOE, Texas Red and CY5 dyes), 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
- 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 FAM, HEX/JOE, Texas Red and CY5 dyes. The below results were studied with Bio-Rad CFX96. The threshold values for all dyes were set to 300-1000*, 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 CY5 dye. The C_T value of internal controls should be **$20 \leq C_T \leq 28$**. 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.

* Depending on the DNA isolation system, the threshold value on CFX96 instruments can be adjusted within this range.

Amplification plots of mutations can be analysed by FAM, HEX/JOE and Texas Red dyes. 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 related mutation (Table 3 and 5).

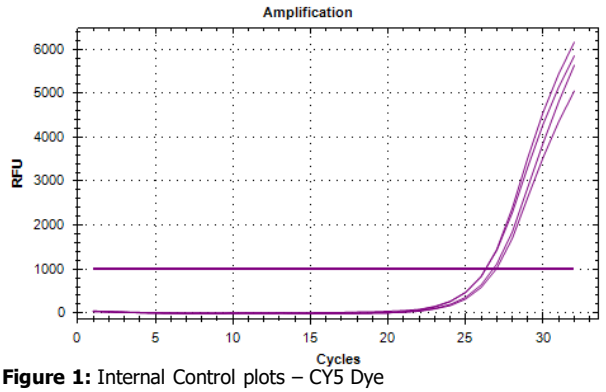

Figure 1: Internal Control plots – CY5 Dye

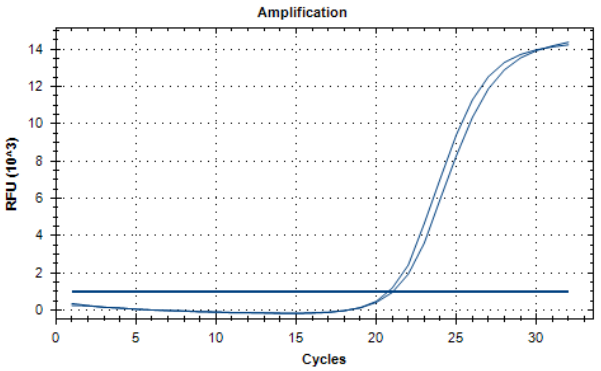

Figure 2: JAK-2 Exon 12 Positive Result- FAM dye - Mix1 and Mix3

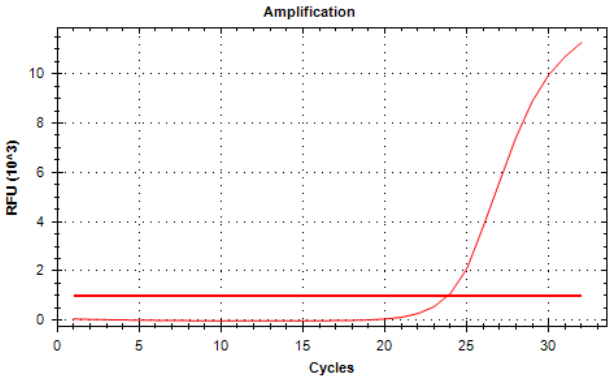

Figure 3: JAK-2 V671F Positive Result- Texas Red dye - Mix 4

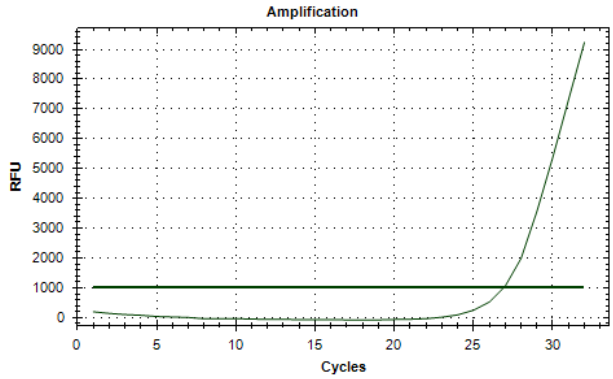

Figure 4: W515K Positive Result- HEX/JOE dye - Mix 4

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Mutations List for CalR

Mix 1 (Texas Red)	c.1111_1113delGAA (p.E372delE), c.1116_1116delA (p.D373fs*57), c.(1117-1119)ins (p.(D373_?)Xfs?), c.(1119_1121)ins? (p.(A352_*)Xfs*?), c.1122_1122delG (p.K375fs*55), c.1120_1122delAAG (p.K375delK), c.1090_1123del34 (p.E364fs*55), c.1122_1123GA>TTGT (p.K374fs*57), c.1120_1123delAAGA (p.K374fs*55), c.1091_1124del34 (p.E364fs*55), c.1113_1125>TTGTCT (p.E371fs*57), c.1122_1125delGAAA (p.K374fs*55), c.1123_1125AAA>TGTTT (p.K375fs*56), c.1123_1125AAA>TTTTGTTT (p.K375fs*57), c.1120_1125AAGAAA>TCTTGTCT (p.K374fs*57), c.1120_1125AAGAAA>TGCGT (p.K374fs*56), c.1125_1125delA (p.K375fs*55), c.1125_1126ins11 (p.R376fs*58), c.1120_1126AAGAAC>TACGTA (p.K374fs*56), c.1120_1126AAGAAC>TTCTTGTCTTCTTGTCTT (p.K374fs*60)
Mix 2 (Texas Red)	c.1151_1154ACAA>TTTGTC (p.D384fs*47), c.1143_1154>TCCTTGTC (p.E381fs*48), c.1147_1154GAGGACAA>TGTC (p.E383fs*46), c.1153_1154AA>TGTC (p.K385fs*46), c.1151_1154ACAA>TATGTC (p.D384fs*47), c.1151_1154ACAA>GCAATTGTC (p.D384fs*48), c.1153_1154AA>TCTTGTC (p.K385fs*47), c.1154_1154A>TTTGTC (p.K385fs*47), c.1154_1154A>TATGTC (p.K385fs*47), c.1150_1154GACAA>TGTC (p.D384fs*46), c.1154_1154A>TCTGTC (p.K385fs*47), c.1154_1155insGTGTC (p.E386fs*46), c.1154_1155insATGTC (p.E386fs*46), **c.1154_1155insTTGTC (p.K385fs*47)(Type 2),** c.1155_1156insTGTCG (p.E386fs*46)
Mix 3 (Texas Red)	c.1129_1135AAAGAGG>CTTTGCGTA (p.K377fs*54), c.1137_1138insAG (p.E380fs*38), c.1129_1138del10 (p.K377fs*50), c.1129_1139>CTCTGTC (p.K377fs*52), c.1129_1139>CTCTGCCTCC (p.K377fs*53), c.1140_1140delG (p.E381fs*49), c.1139_1140insTC (p.E380fs*51), c.1145_1146insGACGC (p.E383fs*49), c.1135_1147del13 (p.E379fs*47), c.1142_1151AGGCAGAGGA>CGGCATGTC (p.E381fs*49), c.1135_1152>CCTCCTCTTTGTCT (p.E379fs*50), c.1131_1152del22 (p.E378fs*45), c.1138_1212>AGGAGGCAGAGG ACAAGGAGGATGATGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGATGA (p.E380fs*62), c.1139_1215>CAAGGAGGA TGATGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGTTGA (p.E380fs*57), c.1143_1220>GCAGAGGACAAGGAGGATGA TGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGATG (p.A382fs*57)
Mix 1 and/or Mix 3 (Texas Red)	c.1127_1129GCA>TTTGC (p.R376fs*55), c.1120_1129del10 (p. K375fs*52), c.1096_1129del34 (p.R366fs*56), c.1120_1131>TGCGT (p.K374fs*54), c.1098_1131del34 (p.L367fs*52), c.1127_1132GCAAAG>TTTGC (p.R376fs*54), c.1100_1133del34 (p.L367fs*52), c.1124_1133del10 (p.K375fs*53), c.1122_1134del13 (p.K375fs*51), c.1100_1134>A (p.L367fs*52), c.1101_1134del34 (p.K368fs*51), c.1102_1135del34 (p.K368fs*51), c.1118_1136del19 (p.D373fs*51), c.1107_1137del 31 (p.E370fs*50), c.1102_1137>GA (p.K368fs*51), c.1104_1137del34 (p.E369fs*50), c.1092_1137del46 (p.Q365fs*50), c.1102_1137>CA (p.K368fs*51), c.1093_1138del46 (p.Q365fs*50), c.1105_1138del34 (p.E369fs*50), c.1120_1138del19 (p.K374fs*50), c.1094_1139del46 (p.Q365fs*50), c.1103_1139del37 (p.K368fs*50), c.1095_1140del46 (p.L367fs*48), c.1122_1140del19 (p.K375fs*49), c.1120_1140>TCTTGTCT (p.K374fs*52), c.1110_1140del31 (p.E371fs*49), c.1112_1142del31 (p.E371fs*49), c.1090_1141del52 (p.E364fs*49), c.1089_1141del53 (p.E364fs*7), c.1111_1141del31 (p.E371fs*49), c.1099_1141del43 (p.L367fs*49), c.1122_1141>A (p.K375fs*49), c.1111_1142>T (p.E371fs*49), c.1091_1142del52 (p.E364fs*46), c.1080_1143>GGAAGAAGACAA (p.Q361fs*52), c.1093_1143del51 (p.Q365_E381del), c.1126_1144del19 (p.R376fs*48), c.1108_1144del37 (p.E370fs*48), c.1114_1144del31 (p.E372fs*48), c.1102_1145>AGGAGGAGGG (p.K368fs*51), c.1109_1145del37 (p.E371fs*47), c.1118_1145>CGTTTA (p.D373fs*50), c.1115_1145del31 (p.D373fs*47), c.1125_1146del22 (p.K377fs*46), c.1116_1146del31 (p.D373fs*47), c.1101_1146del46 (p.K368fs*47), c.1100_1145del46 (p.L367fs*48), c.1111_1147del37 (p.E371fs*47), c.1104_1147>GAGGAGGCAA (p.E369fs*50), c.1104_1148>GAGGAGGCAGT (p.E369fs*50), c.1121_1148del28 (p.K374fs*47), c.1103_1148del46 (p.K368fs*47), c.1101_1152del52 (p.K368fs*45), c.1103_1154del52 (p.K368*45), c.1095_1155del61 (p.L367fs*43), c.1104_1155del52 (p.E369fs*44), c.1099_1156del58 (p.L367fs*44), c.1109_1160del52 (p.E370fs*43), c.1100_1160del61 (p.K368fs*42), c.1113_1160del48 (p.E371_E386del), c.1107_1176del70 (p.E370fs*37), c.1099_1159del61 (p.L367fs*43), **c.1092_1143del52 (p.L367fs*46)(Type 1)**
Mix 2 and/or Mix 3 (Texas Red)	c.1130_1154>TCCATCCTTGTC (p.K377fs*49)

Mutation List for Jak2 V617F

Mix 4 (Texas Red)	JAK-2 V617F

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

Mutation List for CSFR

Mix 1 (HEX/JOE)	T615A
Mix 2 (HEX/JOE)	T618I

Mutations List for MPL

Mix 3 (HEX/JOE)	S505N, W515L, W515R
Mix 4 (HEX/JOE)	W515A, W515K

Table 5: List of mutations screened by dyes and mixes

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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.
- Only use in vitro diagnostics.

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

SYMBOLS AND DESCRIPTIONS

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

Table 6: Symbols and descriptions

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. • 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. • 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. • 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. • 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 7: Troubleshooting problems and solutions