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

PRODUCT DESCRIPTION

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].

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; W515L, W515K 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 last page for detailed information about the mutations that can be screened with the kit.

The kit offers sensitivity to detect under 1% mutant allele in background of 99% wild type allele in the related mutations.

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 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.

PRODUCT SPECIFICATION

Each isolated DNA should be tested with four MPN mixes. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR using SNP analyses. The test system is designed by SNP Biotechnology for use with sequence specific primers and probe.

The fluorescence dyes used for mutation analysis are FAM, HEX and Texas Red. Also each mastermix contains an internal control labelled with CY5 dye. Mutations and related dyes can be seen in Table 1.

DNA EXTRACTION

Blood samples should be collected into 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.

The kit system optimized according to SNPure Blood® and MN NucleoSpin® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

SYSTEM CONTENTS

Reagents	20 rxns
• MPN Mix 1	400 µl
• MPN Mix 2	400 µl
• MPN Mix 3	400 µl
• MPN Mix 4	400 µl
• Control DNA*	75 µl

*Control DNA is a synthetic plasmid. Amplification plots of synthetic control DNA may appear slightly different from the sample DNA.

Table 1: Tubes- mutations- dyes.

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

PROCEDURE

- Different test tubes should be prepared for each mix.
- 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 (~10-100 ng) DNA** into each tube**.**
- Run with the programme shown below.

Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

Table 2: PCR conditions.

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

Fluorescent dyes are FAM, TEXAS RED, CY5 and HEX.

This system can be used with;

Bio-Rad CFX96

ABI Prism® 7000/7300/7500/7500 Fast/7900

If you use;

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

DATA ANALYSIS

After the run completed, data are analysed using the channels with FAM, HEX, TEXAS RED and CY5 dyes. The below results were taken with BioRad CFX96. Amplification plots of mutations should be analysed by related dye/dyes (Please check tubes / mutations / dyes table - Table 1).

Threshold baseline value of FAM, HEX, TEXAS RED and CY5 dyes should be adjusted to 1000 (for BioRad CFX96).

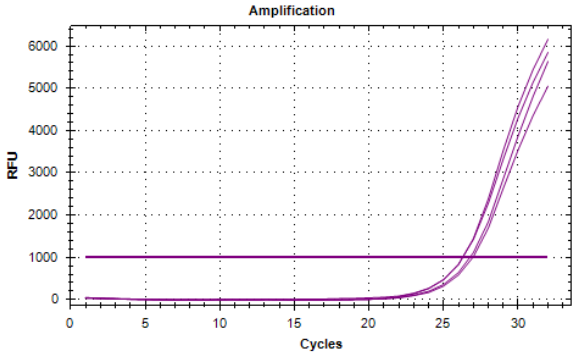

***Figure 1**: Internal controls- CY5 dye*

Internal control amplification plots must be seen in all wells except NTC and has been labelled with CY5 dye. The CT value of internal controls should be between **22 ≤ X ≤ 28**.

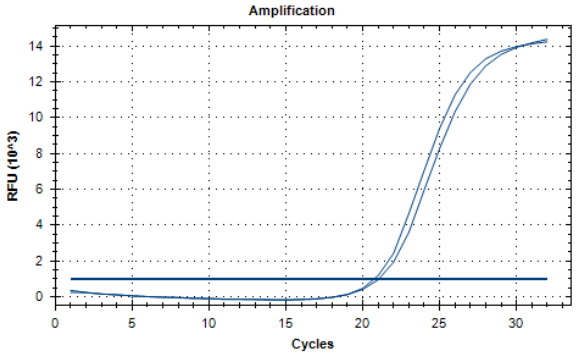

***Figure 2**: JAK-2 Exon 12 Mix1&Mix3 Positive Result- FAM dye*

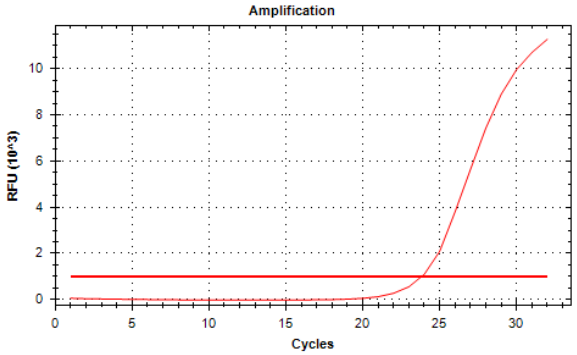

***Figure 3**: JAK-2 V671F Positive Result- TEXAS RED dye/ Mix 4*

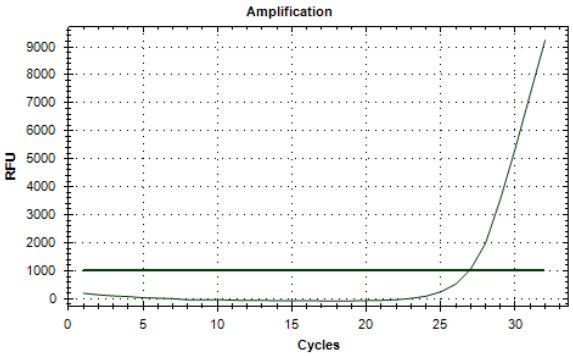

***Figure 4**: W515K Positive Result- HEX dye/ Mix 4*

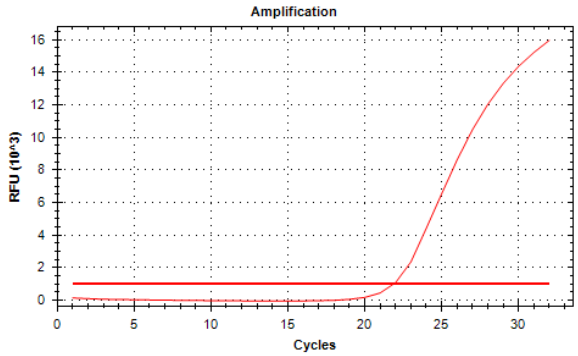

***Figure 5**: CALR Positive Result- TEXAS RED dye/ Mix 2*

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 mutant DNA is very low.

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

REFERENCES

- 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

Mutation List for CalR

	Mutations
Mix 1 (Texas Red Dye)	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 Dye)	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 Dye)	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>AGGAGGCAGAGGACAAGGAGGATGATGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGATGA (p.E380fs*62), c.1139_1215>CAAGGAGGATGATGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGTTGA (p.E380fs*57), c.1143_1220>GCAGAGGACAAGGAGGATGATGAGGACAAAGATGAGGATGAGGAGGATGAGGAGGACAAGGAGGAAGATG (p.A382fs*57)
Mix 1 & Mix 3 (Texas Red Dye)	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 & Mix 3 (Texas Red Dye)	c.1130_1154>TCCATCCTTGTC (p.K377fs*49)

Mutation List for Jak2 Exon12

Tubes	Mutations
Mix 1 (FAM Dye)	F537IK539L, K539L, F533IK539L, H538QK539L, H538DK539LI540S, K539LL545V, H538del, H538-K539delinsL , H538-K539delinsI, H538-K539delinsF, H538-K539del
Mix 4 (FAM Dye)	V536-I546dup11, V536-F547dup12, (V536F,F37-I546dup10), F537-F547dup11, (F537-I546dup10, F547L), (F547L, I540-F547dup8)
Mix 1 & Mix 2 (FAM Dye)	F537-K539delinsK, F537-K539delinsL, F537-K539del
Mix 1 & Mix 3 (FAM Dye)	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 Jak2 V617F

Tubes	Mutations
Mix 4 (Texas Red Dye)	JAK-2 V617F

Mutation List for CSFR

Tubes	Mutations
Mix 1 (HEX Dye)	T615A
Mix 2 (HEX Dye)	T618I

Mutation List for MPL

Tubes	Mutations
Mix 3 (HEX Dye)	W515L
Mix 4 (HEX Dye)	W515K