JAK-2 REAL TIME PCR KIT

Cat. No: 21R-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 real time pcr mastermixes. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR using patented SNP analyses. The test system is designed for use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM. Also each mastermix contains an internal control labelled with HEX dye.

SYSTEM CONTENTS

Reagents	20 rxns	50 rxn
• JAK-2 Wild-Type PCR mastermix	400 µl	1000 µl
• JAK-2 Mutant PCR mastermix	400 µl	1000 µl
• Control DNA	30 µl	60 µl

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.

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 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 filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube**.**
- Run with the programme shown below.

Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

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

Fluorescent dyes are FAM and HEX.

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.

DATA ANALYSIS

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

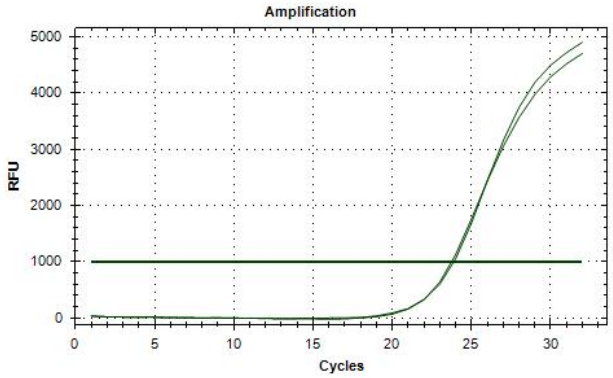

Figure 1: Internal Control plots – HEX Dye

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX dye.

The CT value of internal controls should be **20 ≤ X ≤ 28**.

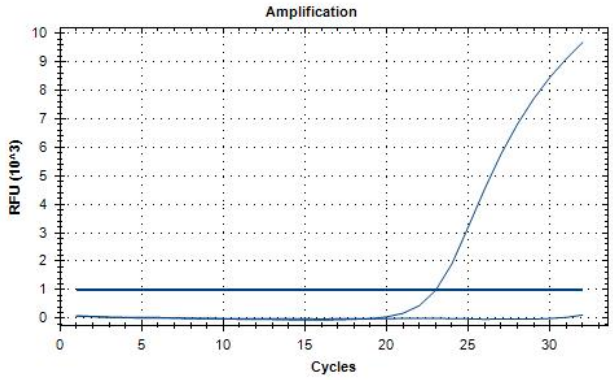

Figure 2: Wild type sample (only amplified with wild type mix) – FAM Dye

Amplification plots of mutations can be analysed by FAM dye.

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

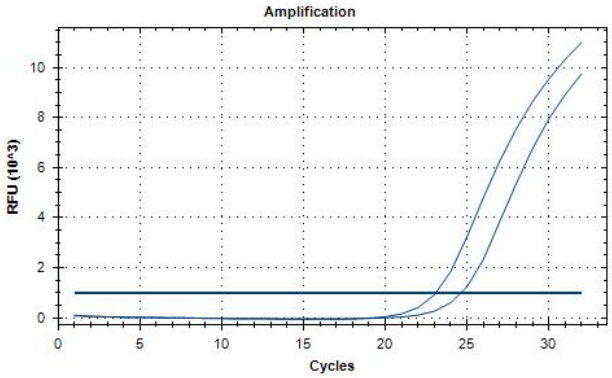

Figure 3: JAK-2 positive sample
(amplified both with wild type and mutant mix) – FAM Dye

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.