THROMBOPHILIA MULTIPLEX REAL TIME PCR KIT B

(6 MUTATIONS)

Cat. No: 10R-20-06B

PRODUCT DESCRIPTION

In Thrombophilia, blood has an increased tendency to form potentially dangerous clots. Hereditary defects in one or more of the clotting factors can cause to excessive blood clot formation called thrombosis. Thrombophilia Multiplex Real Time PCR Kit includes; FII Prothrombin, FV Leiden, FXIII Val34Leu, MTHFR 677, MTHFR 1298 and PAI-1 4G/5G 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 to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Each isolated DNA should be tested with Mix 1, Mix 2, Mix 3 and Mix 4. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR. The test system is designed for use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM, HEX/JOE and Texas Red. Also each mastermix contains an internal control labelled with CY5 dye.

SYSTEM CONTENTS

Reagents	20 rxns	50 rxns
• TRP-6B Mix 1	400 µl	1000 µl
• TRP-6B Mix 2	400 µl	1000 µl
• TRP-6B Mix 3	400 µl	1000 µl
• TRP-6B Mix 4	400 µl	1000 µl
• Control DNA	45 µl	90 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 (>4X) 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 SNPure Blood® and MN NucleoSpin ® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

MUTATION / DYE TABLE

Table 1 : Tubes- mutations- dyes.

Tubes	Mutations	Dyes
Mix 1	FII Wild Type	FAM
	FV Leiden Wild Type	HEX/JOE
	677 Wild Type	TEXAS RED
	Internal Control	CY5
Mix 2	FII Mutant	FAM
	FV Leiden Mutant	HEX/JOE
	677 Mutant	TEXAS RED
	Internal Control	CY5
Mix 3	1298 Wild Type	FAM
	FXIII Wild Type	HEX/JOE
	PAI – 1 5G	TEXAS RED
	Internal Control	CY5
Mix 4	1298 Mutant	FAM
	FXIII Mutant	HEX/JOE
	PAI – 1 4G	TEXAS RED
	Internal Control	CY5

PROCEDURE

- Different 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 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

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

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

If you use;

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

This system can use with;

ABI Prism ® 7500/7500 Fast

Bio-Rad CFX96

DATA ANALYSIS

After the run is completed data are analysed using the software with HEX (JOE), TEXAS RED, CY5, and FAM dyes. The below results were studied with ABI 7500.

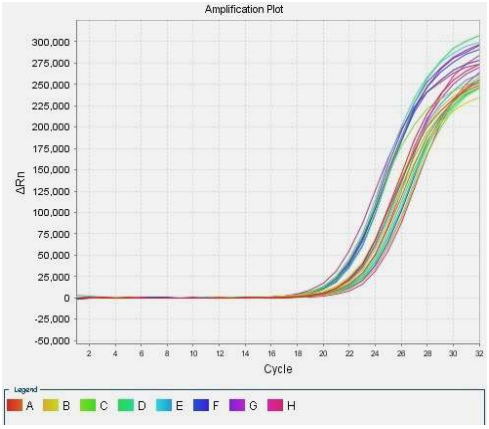

Figure 1: Internal Control plots – CY5 Dye

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 **21 ≤ X ≤ 26**.

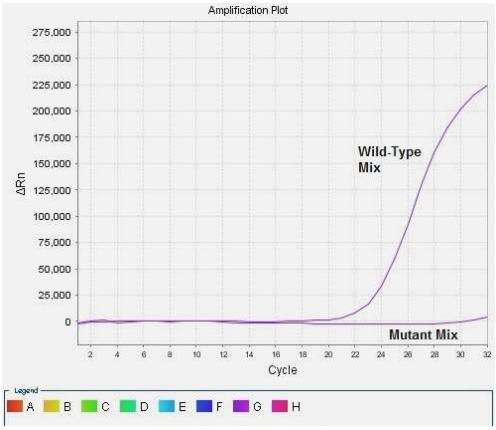

Figure 2: FII Prothrombin Wild Type – FAM Dye

Amplification plots of mutations can be analysed by related dye*. The CT value should be between **21 ≤ CT ≤ 26**. These values are optimised according to the SNPure® Blood DNA Isolation Kıt and MN NucleoSpin ® Blood DNA Isolation Kıt. CT values may vary ±2/3 cycle according to the DNA isolation protocol.

- Homozygote wild-type sample gives amplification signal only with wild-type mastermix.
- Heterozygote sample gives amplification signal both with wild-type and mutant mastermixes.
- Homozygote mutant sample gives amplification signal only with mutant mastermix.
- The diffrence of the CT value wild-type and mutant amplification plots should be **≤3** for heterozygote mutant sample. It is **3 < CT Difference < 6,** test should be repeated. If it is **≥6** the lower peak should be considered non-specific. **Please check tubes / mutations / dyes table (table 1).*

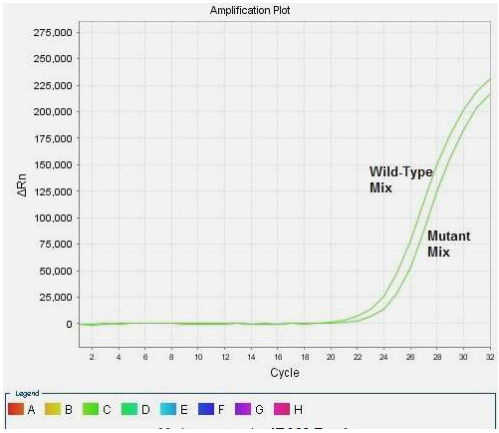

Figure 3: FV Leiden Heterozygote – JOE Dye

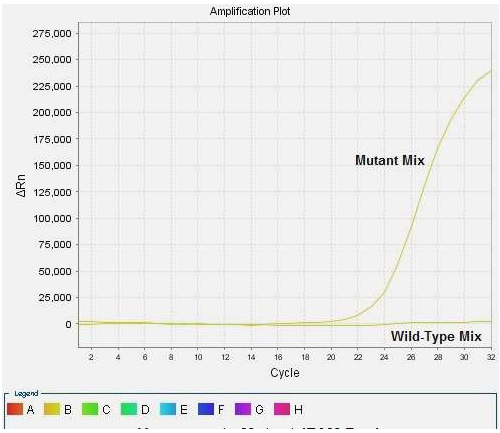

Figure 4: 677 Homozygote mutant – TEXAS RED Dye

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Absence/Deficiency of Hot Start Taq DNA Polymerase
- Sample is containing DNA inhibitor(s)

If plots start late,

- DNA quality is not good.
- The amount of DNA is not enough.
- Sample is containing parcial DNA inhibitor(s)

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.