FMF MULTIPLEX REAL TIME PCR KIT

(13 MUTATIONS)

Cat. No: 11R-20-13

PRODUCT DESCRIPTION

Familial Mediterranean Fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and polyserositis. It affects primarly people of Mediterranean, mostly non-Ashkenazi Jews, Araps and Turks. Kit analysis thirteen mutations, which has been identified in exon 2; E148Q, E148V, in exon 3; P369S, in exon 5; F479L and in exon 10; M680I (G/C-A), I692DEL, M694I, M694V, K695R, V726A, A744S, R761H. Kit is covering 97% mutation rate of FMF in the Anatolian, Middle East countries and many other countries.

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 SNP analyses. The test system is designed to use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM and HEX/JOE. Also each mastermix contains an internal control labelled with CY5 dye. Mutations and related dyes can be seen in Table 1.

SYSTEM CONTENTS

Reagents	20 rxns	50 rxns
• Mix 1	400 µl	1000 µl
• Mix 2	400 µl	1000 µl
• Mix 3	400 µl	1000 µl
• Mix 4	400 µl	1000 µl
• Mix 5	400 µl	1000 µl
• Mix 6	400 µl	1000 µl
• Mix 7	400 µl	1000 µl
• Mix 8	400 µl	1000 µl
• Mix 9	400 µl	1000 µl
• Mix 10	400 µl	1000 µl
• Mix 11	400 µl	1000 µl
• Mix 12	400 µl	1000 µl
• Control DNA*	75 µl	150 µl

*Control DNA is a synthetic plasmid containing some of the mutation regions. Expected results for synthetic control DNA should be I692del Wild Type, M694I Wild Type, M680I Homozygote Mutant, K695R Wild Type, A744S Wild Type, M694V Homozygote Mutant, V726A Homozygote Mutant and R761H Homozygote Mutant. Amplification plots of synthetic control DNA may appear slightly different from the sample DNA.

Table 1 : Tubes- mutations- dyes.

Tubes	Mutations	Dyes
Mix 1	I692DEL Wild Type	FAM
	A744S Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 2	I692DEL Mutant Type	FAM
	A744S Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 3	P369S Wild Type	FAM
	M694V Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 4	P369S Mutant Type	FAM
	M694V Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 5	E148Q Wild Type	FAM
	V726A Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 6	E148Q Mutant Type	FAM
	V726A Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 7	F479L Wild Type	FAM
	M694I Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 8	F479L Mutant Type	FAM
	M694I Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 9	M680I Wild Type	FAM
	E148V Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 10	M680I Mutant Type	FAM
	E148V Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 11	K695R Wild Type	FAM
	R761H Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 12	K695R Mutant Type	FAM
	R761H Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5

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.

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.

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
62 °C	1 Min.	

Fluorescent dyes are FAM, CY5 and HEX/JOE.

If you use;

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

This system can be used with;

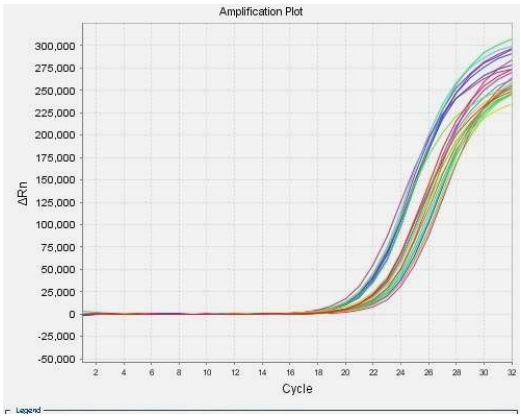
Bio-Rad CFX96

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

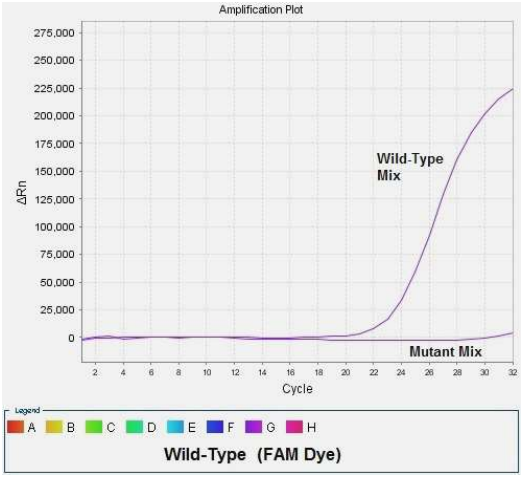
DATA ANALYSIS

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 ABI7500.

An analysis table (table 2) can be found for easy evaluation, at the end of the protocol.



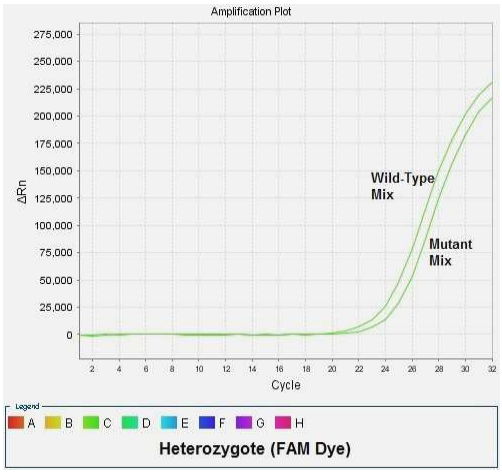
Internal control amplification plots must be seen in all wells except NTC and has been labelled with CY5 dye. The CT value of internal controls should be **22 ≤ X ≤ 26**.

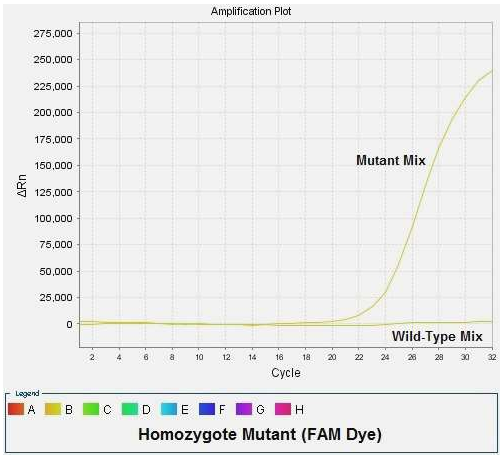


Amplification plots of mutations can be analysed by related dye*. The CT value should be between **21 ≤ CT ≤ 26**. These values are optimised according to the SNPure® Blood DNA Isolation Kit and MN NucleoSpin ® Blood DNA Isolation Kit. CT values may vary ±2/3 cycle according to the DNA isolation protocol.

- 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 ≤ 4**, test should be repeated. In cases where the Ct value difference is **> 4**, the result can be given as normal.

**Please check tubes / mutations / dyes table (table 1).*





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 DNA is not enough.

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.

REFERENCES

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SNP Biotechnology

Well	Sample												
A													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Int. Control / *CY5*		Empty		Int. Control / *CY5*		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
B													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
C													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
D													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
E													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
F													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
G													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
H													
	I692DEL / *FAM*		P369S / *FAM*		E148Q / *FAM*		F479L / *FAM*		M680I / *FAM*		K695R / *FAM*		
	A744S / *JOE-HEX*		M694V / *JOE-HEX*		V726A / *JOE-HEX*		M694I / *JOE-HEX*		E148V / *JOE-HEX*		R761H / *JOE-HEX*		
	Empty		Empty		Empty		Empty		Empty		Empty		
	Int. Control / *CY5*		Int. Control / *CY5*				Int. Control / *CY5*				Int. Control / *CY5*		
	Mix1 (WT)	Mix2 (MT)	Mix3 (WT)	Mix4 (MT)	Mix5 (WT)	Mix6 (MT)	Mix7 (WT)	Mix8 (MT)	Mix9 (WT)	Mix10 (MT)	Mix11 (WT)	Mix12 (MT)	

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