OCTOBIO

OCTO Health RT-PCR FMF All in One Kit

Catalog #	Description
RT-FMF-ALL-25	Real-Time PCR FMF All in One Kit, 25 reactions
RT-FMF-ALL-50	Real-Time PCR FMF All in One Kit, 50 reactions

IVD

CE

Introduction

Familial Mediterranean Fever (FMF) is a genetic disorder primarily observed in populations of Mediterranean origin. It affects approximately 1-3 per 1,000 individuals, with regional variations in prevalence. FMF is characterized by recurrent episodes of fever, abdominal pain, chest pain, and joint inflammation, which can significantly impact quality of life. Early and accurate diagnosis, often through the detection of specific mutations, is critical for effective management and prevention of complications such as amyloidosis. *Octo Health RT-PCR FMF All in One Kit* is a comprehensive mutation detection assay. By targeting mutations in exons 2, 3, 5, and 10, the kit can genotype a total of 28 distinct mutations. In addition, the kit is capable of identifying compound mutations.

	LEGACY NAME	NUCL. CHANGE	AA CHANGE	RS #
1	M694V	c.2080A>G	p.Met694Val	rs61752717
2	E148Q	c.442G>C	p.Glu148Gln	rs3743930
3	E148V	c.443A>T	p. Glu148Val	rs104895076
4	V726A	c.2177T>C	p.Val726Ala	rs28940579
5	M680I (G/C)	c.2040G>C	p.Met680Ile	rs28940580
6	M680I (G/A)	c.2040G>A	p.Met680Ile	rs28940580
7	P369S	c.1105C>T	p.Pro369Ser	rs11466023
8	R761H	c.2282G>A	p.Arg761His	rs61732874
9	A744S	c.2230G>T	p.Ala744Ser	rs61732874
10	M694I	c.2082G>A	p.Met694Ile	rs28940578
11	K695R	c.2084A>G	p.Lys695Arg	rs104895094
12	E167D	c.501G>C	p.Glu167Asp	rs104895079
13	F479L	c.1437C>G	p.Phe479Leu	rs104895083
14	I692Del	c.2076_2078del	p.Ile692del	rs104895093
15	R408Q	c.1223 G > A	p.Arg408Gln	rs11466024
16	R42W	c.124C>T	p.Arg42Trp	rs61754767
17	E84K	c.250G>A	p.Glu84Lys	rs150819742
18	E230K	c.688G>A	p.Glu230Lys	rs104895080
19	E230Q	c.688G>C	p.Glu230Gln	rs104895080
20	T267I	c.800C>T	p.Thr267Ile	rs104895081
21	P283L	c.848C>T	p.Pro283Leu	rs104895119
22	G304R	c.910G>A	p.Gly304Arg	rs75977701
23	R354W	c.1060C>T	p.Arg354Trp	rs104895116
24	I591T	c.1772T>C	p.Ile591Thr	rs11466045
25	R653H	c.1958G>A	p.Arg653His	rs104895085
26	P350R	c.1049C>G	p.Pro350Arg	rs28940582
27	S179I	c.535G>T	p.Ser179Ile	rs121908479
28	L110P	c.329T>C	p.Leu110Pro	rs114660235

Kit Contents

	CONTENT	25 REACTIONS	50 REACTIONS
1	Master Mix 1	450 µl	900 µl
2	Master Mix 2	450 µl	900 µl
3	Master Mix 3	450 µl	900 µl
4	Master Mix 4	450 µl	900 µl
5	Master Mix 5	450 µl	900 µl
6	Master Mix 6	450 µl	900 µl
7	Master Mix 7	450 µl	900 µl
8	Master Mix 8	450 µl	900 µl
9	Control Mix	200 µl	400 µl

The Control Mix contains synthetic wild-type and mutant sequences for each parameter specified in the table. All controls are expected to produce heterozygous profiles during the melt-curve analysis

Required Equipment and Materials:

- Compatible RT-PCR Devices*

  Biorad CFX96 and Opus, Qiagene Rotorgene, Quantstudio Real-Time PCR Systems, Roche LightCycler 480 & 96, Mic qPCR Cycler, Corbett Realtime PCR, Slan Realtime PCR, Molarray MA6000, Himedia Insta Q96, Q48 and Q48M
- DNA Isolation (Manual or automated system)
- Vortex and Centrifuge
- Pipette Set (0.5-10µl and 10-100µl)
- Sterile Pipette Tips
- Cold Block
- PCR tubes or plates compatible with the respective Real-Time PCR Device
- +4°C Refrigerator
- Sterile Gloves

* *Please contact for other compatible devices*

Storage Conditions

- All components of the kit should be stored at -20°C until the expiration date.

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- Kit components should undergo freeze-thaw cycles a maximum of 5 times. If more than 5 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.

Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice or cooling rack.
- Once thawed, liquid materials should be mixed well by pipetting.

Preparation of Reactions:

1. Preparation of 5 tubes is required for each sample. Reaction volumes are specified in the table. The same tubes should be prepared for the NTC.

Content	Amount (µl)
PCR Master Mix	18
DNA Isolate / NTC	2
TOTAL	20

Real – Time PCR Protocol

Cycle	Temperature (°C)	Time (Seconds)	Fluorescent Dye
1	95 °C	300 s	
50	95 °C	10 s	
	60 °C	45 s	FAM, HEX, ROX, CY5
1	95 °C	180 s	
	55 °C	60 s	
Melt Analysis			
1	35 °C	1 s	
Initial Temp. (°C)	35 °C	**Ramp Rate (°C /sec)**	0.3
End Temperature	85 °C	**Hold (sec)**	1 s

Data Analysis – Interpretation of Results

- After the Real-Time PCR stage, analysis begins by selecting the Melt Analysis option.
- Each tube must be analyzed separately.
- The analysis should be performed based on the presence or absence of peaks at the specified temperatures for each parameter.
- OctoClario™ Analysis Software can be used for automated analysis. Refer to the Software User Guide for program details.

TUBE 1	Emission Ch.	Mutant Tm	Wild Type Tm
E148Q	ROX	62°C	73°C
E148V		67°C	
R761H	CY5	54°C	62°C

TUBE 2	Emission Ch.	Mutant Tm	Wild Type Tm
F479L	ROX	55°C	62°C
R408Q	CY5	60°C	71°C
R354W	FAM	50°C	60°C

TUBE 3	Emission Ch.	Mutant Tm	Wild Type Tm
P369S	CY5	57°C	63°C
P350R	FAM	60°C	65°C
E230K/E230Q	ROX	54°C	62°C

TUBE 4	Emission Ch.	Mutant Tm	Wild Type Tm
M694V	ROX	64°C	58°C
M694I		53°C	
K695R		55°C	
I692Del		49°C	
M680I(G/C)	CY5	47°C	59°C
M680I(G/A)		48°C	

TUBE 5	Emission Ch.	Mutant Tm	Wild Type Tm
A744S	FAM	49°C	59°C
V726A	CY5	65°C	60°C
T267I	ROX	62°C	56°C

TUBE 6	Emission Ch.	Mutant Tm	Wild Type Tm
L110P	ROX	58°C	53°C
E167D	CY5	65°C	56-58°C
R42W	FAM	58°C	65°C
E84K	HEX	45°C	60°C

TUBE 7	Emission Ch.	Mutant Tm	Wild Type Tm
S179I	CY5	62°C	70°C
I591T	ROX	48°C	56°C
R653H	FAM	60°C	52°C

TUBE 8	Emission Ch.	Mutant Tm	Wild Type Tm
P283L	ROX	64°C	58°C
G304R	CY5	62°C	57°C

Troubleshooting

1. If no melting peaks are observed in both controls and samples,
   - *Possible causes:* incomplete homogenization of the master mix; annealing/extension temperature set too high; insufficient or degraded template DNA.
     - *Solutions:* pipette and briefly centrifuge each reagent tube before setup; verify thermal-cycler temperatures strictly match IFU; repeat DNA extraction and check the quality of extracted DNA by fluorometry.
2. If control or sample melt-curve peaks are markedly lower in fluorescence than expected,
   - *Possible causes:* dye depletion due to ambient light exposure or prolonged room-temperature storage; expired or improperly stored reagents.
   - *Solutions:* protect master mix and reaction plates from light and keep on ice until use; check kit lot's expiration and storage logs; replace with a fresh kit if needed.
3. If melt peaks appear at temperatures outside the ±1 °C window of the expected T_m,
   - *Possible causes:* incorrect ramp rate or hold time during melt acquisition; nonspecific amplicons (e.g. primer-dimers).
   - *Solutions:* confirm melt-curve program parameters exactly match IFU.
4. If melt-curve derivative baselines are noisy or drift upward,
   - *Possible causes:* insufficient calibration of the instrument's optical modules; wells at plate edges subject to temperature lag.
   - *Solutions:* perform calibration with manufacturer's reference plate; avoid using outermost wells for critical controls or include a software-specified "edge-correction."
5. If sample genotyping calls fail QC despite clean control results,
   - *Possible causes:* sample inhibitors (e.g. heme, guanidine) carried over from extraction; pipetting errors.
   - *Solutions:* repeat extraction using a spin-column kit with an inhibitor-removal step; employ multichannel pipette with filtered tips and include replicate wells.
6. If unexpected peaks appear in the no-template control (NTC),
   - *Possible causes:* airborne DNA contamination.
   - *Solutions:* enforce unidirectional workflow (pre-PCR vs. post-PCR areas).

Tip: Always consult your instrument's maintenance log and ensure that filter sets and heating blocks are within specification before attributing anomalies to the chemistry.

Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• Do not use expired kit. • Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test. • Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly. • Prepare the reaction mixture quickly on ice or a cold block. • Establish two separate work areas, one for isolation and the other for PCR. • Avoid transferring pipettes, tubes, and other working materials between the work areas. • Always use filtered sterile pipette tips. • Use separate lab coats and gloves for each work area.

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**



:Number of Reactions


: Expiration Date



: Lot Number



: Reference Number


: Temperature Limit


: Refer to the User Manual


: Manufacturer


: Keep Away From Direct Sunlight

Octo Biotechnology R&D Inc.
REŞİTPAŞA Mah. KATAR Cad. ARI 4 Binası No: 2 /50/6 SARIYER / İSTANBUL ***T:*** 0090 212 286 1368 | +90 555 331 7254
info@octobiotechnology.com www.octobiotechnology.com

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