Instructions For Use

CE IVD *For In Vitro Diagnostic Use*

Store at -25ºC to -15ºC

REF FMF.5-RTT50

Σ 50

geneMAP™ FMF 5 Mutation Detection Kit

For Realtime PCR Insturment

Kit for detection of E148Q, M694V, V726A, M680I (G/C), P369S mutations in human MEFV gene.

Valide :

* Biorad® CFX96, Real-time PCR System (Bio-Rad)
* Life Technologies ABI Prism® - 7500, Step-One & QuantStudio Series
* Roche, LightCycler® 480 II, Cobas Z480
* BioMolecular Systems, MicPCR

Contents

1. Intended Use

The kit detects **E148Q, M694V, V726A, M680I (G/C), P369S** mutations which are frequently observed in the MEFV gene.

2. Safety Instructions & General Warnings

- This kit must be used strictly in accordance with the instructions provided in this manual, and only in combination with validated reagents and instruments. Any off-label use of this product, as well as any modification of its components, will nullify Genmark's liability.
- Obey proper laboratory safety protocols when working with chemicals and specimens.
- The protocol can be performed by only professional and trained personal.
- Perform the protocol in a well-ventilated and well-lit environment.
- Store the kit and its components within recommended temperature range in de-frost refrigerators. Do not use no-frost refrigerators.
- Avoid skin contact with any of the reagents in the kit
- Wipe workspace surfaces with 10% bleach followed by 70% alcohol.
- Instruments may exhibit performance variations due to differences in electrical currents and power outlets, as well as the effects of maintenance and calibration. It is the responsibility of the user to ensure that all instruments are properly maintained and regularly calibrated according to the manufacturer's guidelines. Genmark disclaims responsibility for any performance issues arising from improper maintenance, calibration, or variations in electrical supply.
- All biological specimens should be handled as potentially infectious, following standard precautions. For guidelines on specimen handling, refer to the World Health Organization.
- Consult environmental waste personnel for proper disposal of used plates, consumables, and reagents, considering federal and local hazardous waste regulations. Check local and national disposal requirements.

3. Reagents and equipments

3.1 Package Contents

Reagents contained in a kit are sufficient for **50** reactions.

Description	No. of Reactions*	No. of Tubes	Vol. Per Tube	Color of Caps	Contents
2X Master Mix (with Evagreen)	140	1	1100 µl	Amber	• DNA polymerase with UDG • Buffer containing dNTPs • Green Intercalating Dye
2X Master Mix (with Evagreen)	70	1	550 µl	Amber	• DNA polymerase with UDG • Buffer containing dNTPs • Green Intercalating Dye
FMF Mix 1 (E148Q-M694V Wildtype)	55	1	110 µl	Yellow	• Amplification and detection reagents • E148Q-M694V Wildtype
FMF Mix 2 (E148Q-M694V Mutant)	55	1	110 µl	Blue	• Amplification and detection reagents • E148Q-M694V Mutant
FMF Mix 3 (V726A-M680I -P369S Wildtype)	55	1	110 µl	Yellow	• Amplification and detection reagents • V726A-M680I -P369S Wildtype
FMF Mix 4 (V726A-M680I -P369S Mutant)	55	1	110 µl	Blue	• Amplification and detection reagents • V726A-M680I -P369S Mutant
Multiplexer Solution	210	1	750 µl	Green	• Buffering Agents • Detergents/Surfactants • Stabilizers

3.2 Handling and Storage

This product is shipped on frozen ice packs or dry ice and may be thawed upon arrival. The contents of the shipment should be frozen at -25°C to -15°C immediately upon receipt. Do not store the kit or any of its components below -25°C as it will adversely affect the performance of the assay.

- Store all unopened components in original containers.
- Centrifuge the tubes before opening.
- The expiration date of each component is printed on each tube label. This product will maintain its performance through this date. Its performance is not guaranteed after the expiration date. Storage and handling of the components of this product under conditions other than those described above may affect the performance of this assay and adversely affect the results.

3.3 Product Stability

This product and its components will maintain performance through the expiration date printed on the labels of each tube given that the storage and handling conditions described above are properly followed.

3.4 Quality Control

The components of this product are manufactured under ISO 9001 and 13485 standards Each batch is tested on an Biorad CFX96 Certificate of Analysis for each batch is available upon request.

3.5 Warning and Precautions

PCR amplification is extremely sensitive to cross-over contamination. It is very important to carry out the preamplification steps (i.e. DNA isolation, PCR reaction preparation) in separate areas, ideally separate rooms with isolated air venting systems. It is especially important that the PCR reaction preparation is carried out in a room with positive air pressure (or laminar flow hood). Additional precautions are necessary when handling DNA and PCR reagents to avoid contamination between samples/reagents:

- Wipe down the work area, pipettors and equipment to be used near the work area with surface decontaminant (20% bleach or equivalent) to eliminate DNA and DNase followed by 70% ethanol.
- Vortex each tube before use.
- Spin down all tubes in picofuge before opening.
- Open each microcentrifuge tube carefully after vortexing/mixing and avoid touching the inside of the lid.
- Use aerosol-resistant (filtered) tips for all pipetting steps to avoid cross-contamination.
- Change pipette tips between all liquid transfers.
- Always wear gloves when handling DNA/reagents and change gloves between the pre-amplification and postamplification steps.
- The flow of tubes, racks, pipettes and other equipment used should be from pre-amplification to postamplification, and never backwards.

4. Required equipment and materials

4.1 Reagents

This kit does not contain reagents for extraction of genomic DNA from blood or buccal swap samples. Genomic DNA from blood or buccal swap can be extracted using common laboratory reagents. The optimal concentration of DNA should be between 20ng/µl and 200 ng/uL.

4.2 Equipment

Equipment required to perform this assay are:

- Real-Time PCR instrument
- Disposable powder-free gloves
- Adjustable pipettes
- Sterile filtered pipette tips (RNase/DNase free)
- Vortex mixer
- Picofuge for 0.2 ml and 2.0 ml microcentrifuge tubes
- 96-well PCR plates (check PCR machine manual for compatible plates)
- Optical sealing film for PCR plates
- 0.5 ml (optional) and 1.5 ml microcentrifuge tubes
- 70% ethanol
- 20% chlorine bleach (or equivalent)

5. Introduction

The MEFV gene provides instructions for making a protein called pyrin (also known as marenostrin). Although pyrin's function is not fully understood, it likely assists in keeping the inflammation process under control. Inflammation occurs when the immune system sends signaling molecules and white blood cells to a site of injury or disease to fight microbial invaders and facilitate tissue repair. When this has been accomplished, the body stops the inflammatory response to prevent damage to its own cells and tissues.

Pyrin is produced in certain white blood cells (neutrophils, eosinophils, and monocytes) that play a role in inflammation and in fighting infection. Pyrin may direct the migration of white blood cells to sites of inflammation and stop or slow the inflammatory response when it is no longer needed. Pyrin also interacts with other molecules involved in fighting infection and in the inflammatory response. Research indicates that pyrin helps regulate inflammation by interacting with the cytoskeleton, the structural framework that helps to define the shape, size, and movement of a cell.

More than 80 MEFV gene mutations that cause familial Mediterranean fever have been identified. A few mutations delete small amounts of DNA from the MEFV gene, which can lead to an abnormally small, nonfunctional protein. Most MEFV gene mutations, however, change one of the protein building blocks (amino acids) used to make pyrin. The most common mutation replaces the amino acid methionine with the amino acid valine at protein position 694 (written as Met694Val or M694V). Among people with familial Mediterranean fever, this particular mutation is also associated with an increased risk of developing amyloidosis, a complication in which abnormal protein deposits can lead to kidney failure.

MEFV gene mutations lead to reduced amounts of pyrin or a malformed pyrin protein that cannot function properly. As a result, pyrin cannot perform its presumed role in controlling inflammation, leading to an inappropriate or prolonged inflammatory response. Fever and inflammation in the abdomen, chest, joints, or skin are signs of familial Mediterranean fever. (1)

6. Technology

Realtime Allel Specific (ASO)-PCR based technology

7. Overview of Procedure

DNA extraction from blood or buccal swap. (required but not provided)

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Realtime PCR

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Analysis of the curves and evaluation

8. Test Protocol

8.1 Thermal Protocol

Temperature	Duration	Cycle	Ramp for LC480	
95 °C	15 min	1	4,4	Realtime PCR Step
95 °C	0:20 sec	10	3,5	
64 °C	0:45 sec		2,2	
95 °C	0:20 sec	22	3,5	
60 °C	0:30 sec		2,2	
72 °C	0:15 sec plate read		3,5	

Melting Curve Step

95°C	0:30 sec	1		Melting Curve Step
50°C	00:30 sec	1		
70°C to 98°C	Increment 0,2°C for 0:05 +plate read	1	Biorad CFX96 (Biorad)	
70°C to 98°C	0.05°C / sec, 12 acquisitions per °C	1	LC480 (Roche)	
70°C to 98°C	70°C to 98°C Ramp percentage:0.2%-0.5%	1	ABI7500, StepOne Series (Life Tech.)	

8.2 Florophore (Dye) Selection

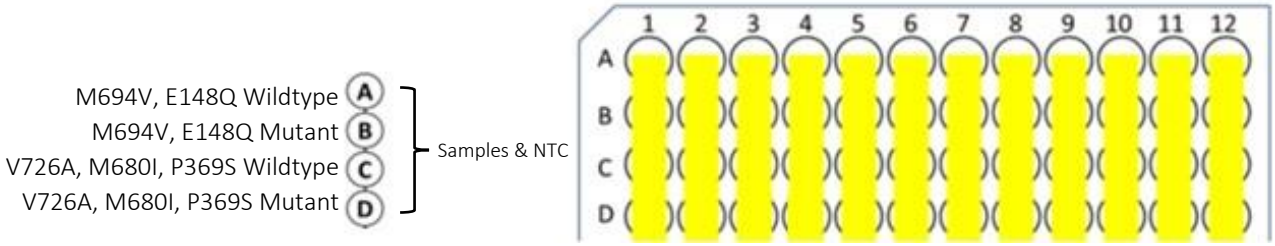
For each sample **FAM or SYBR Green/Eva Green** must be select. Passive referance dye must be select as "none" for ABI series instruments.

8.3 Reagent preperation

The following reagents go into each 15 µl reaction;

Components	Volume
2X Master Mix (with EvaGreen)	7,5
Multiplexer Solution	3,5
Primer Mix (Mix1 or Mix2 or Mix3 or Mix4)	2
DNA (20-100 ng/µL), NTC	2

1. Thaw the necessary primer mixes, multiplexer solution and 2X reaction mix for the run.
2. Mix each tube by vortexing and centrifuge for 5 seconds in picofuge at room temperature.
3. Prepare a master mix for each sample as follows (calculated with 5% overage)
4. Dispense 13 µl of the master mix per well into a single column (A-H) as shown.
5. Add 2 µl of each primer mix to its corresponding well as shown in the following Figure 1.



9. Analysis

The results are evaluated by the presence of PCR amplification curves of the mutant mixes and their melting temperatures. Please review the evaluation chart below for information on each mutation and its melting temperature.

Reagent	Mutation	Expected TM (Temperature Melting)
Mix1	M694V, E148Q Wildtype	83°C-93°C
Mix2	M694V, E148Q Mutant	83°C-93°C
Mix3	V726A, M680I, P369S Wildtype	76°C-83°C-87°C
Mix4	V726A, M680I, P369S Mutant	76°C-83°C-87°C

10. Evaluation of the patients

10.1 Mutation Detection

When study is finished, the amplification and melting curves in each well are checked and mutation status of patients is analyzed according to the melting curve temperatures in the relevant wells.

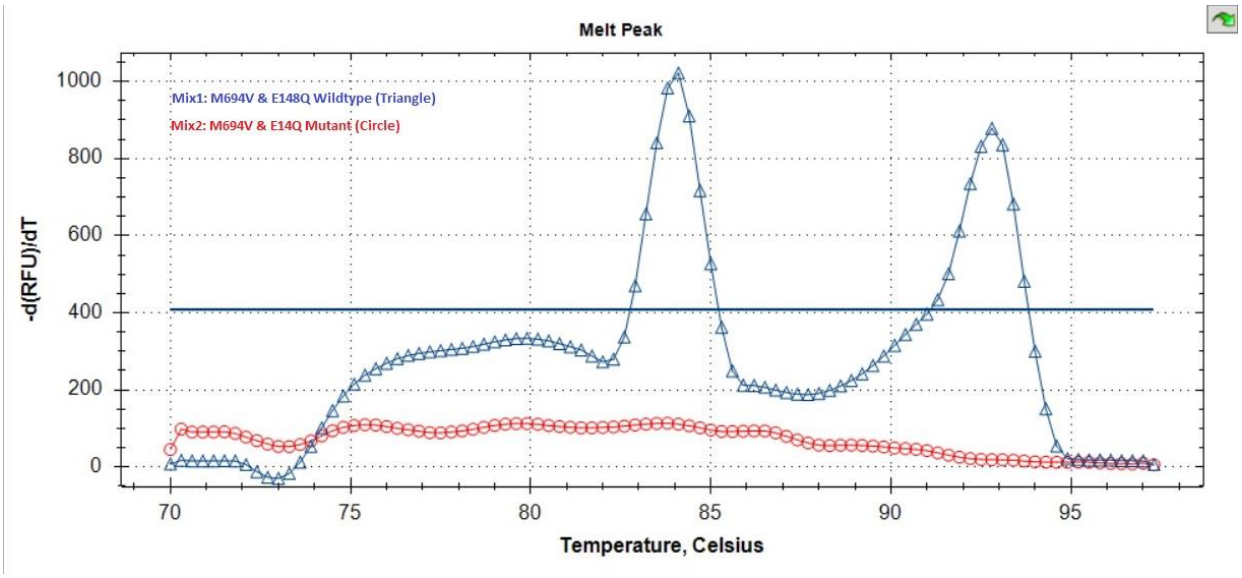
10.2 Mutation Genotyping

The evaluation for each patient is as following table:

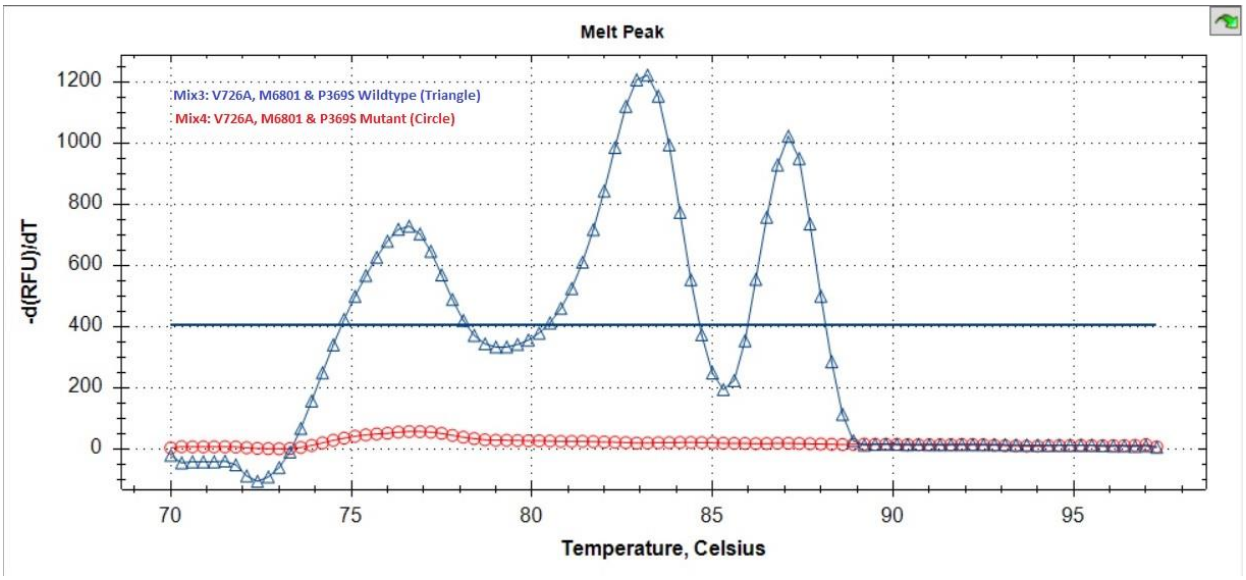
Patient	Mutant Melting Curve	Wildtype Melting Curve	Mutation Status	Genotype
1	-	+	Wildtype	Wildtype
2	+	+	Mutant	Heterozygous
3	+	-	Mutant	Homozygous
4	-	-	İnvalid	İnvalid

11. Example Results

11.1 Wildtype Patient Result (Mix 1,2,3 and 4)

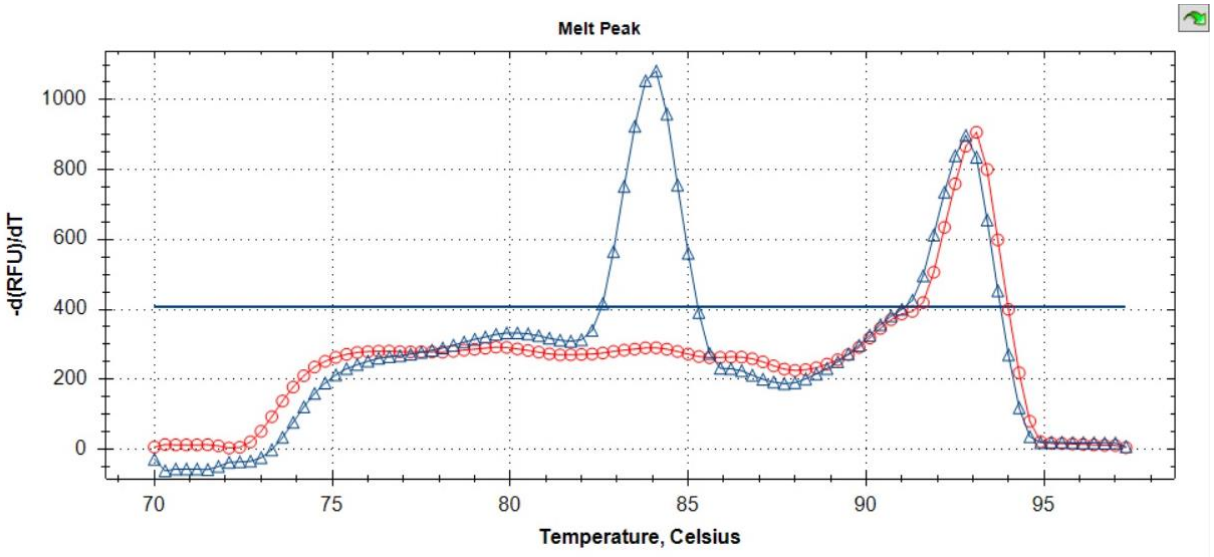


Mix1,2: M694V& E148Q Wildtype.

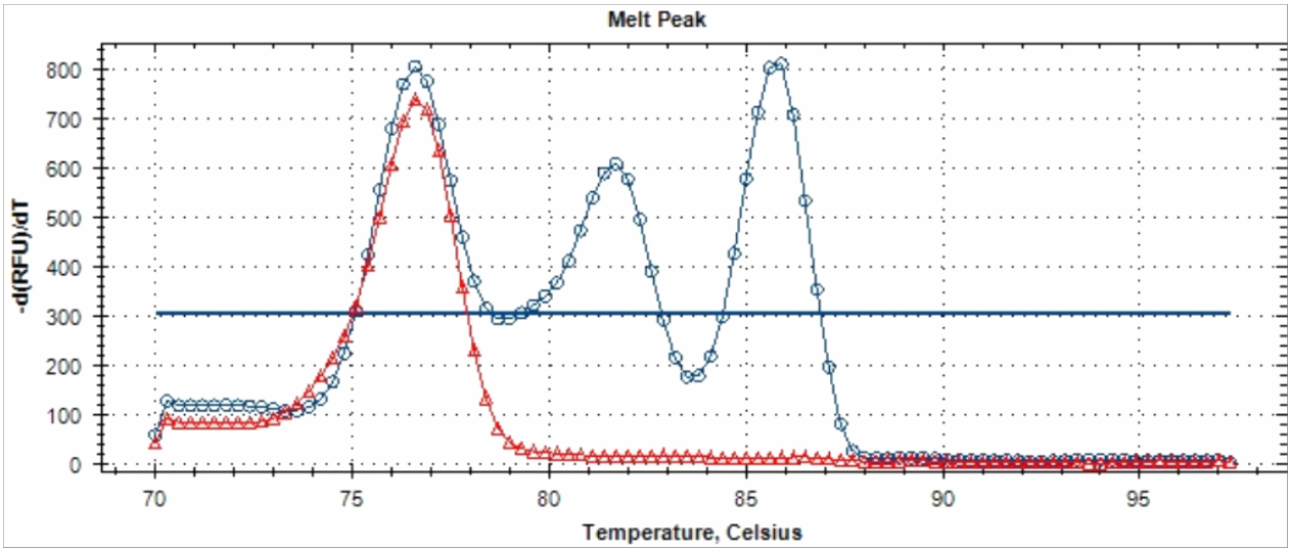


Mix3,4: V726A, M680I & P369S Wildtype.

11.2 Heterozygous patient results

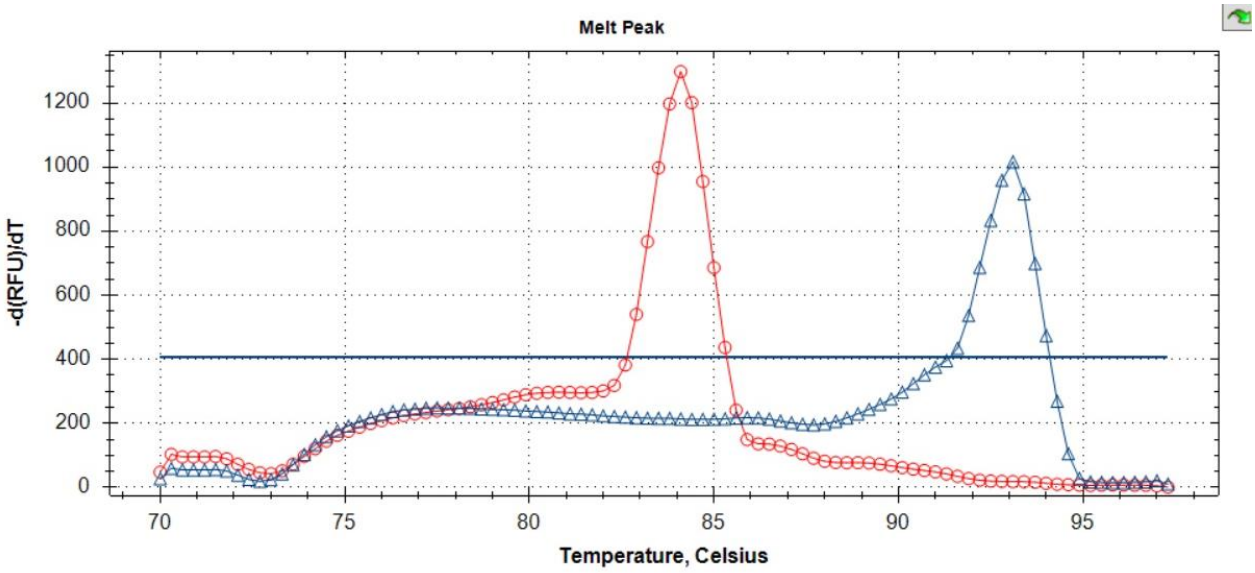


Mix1,2: E148Q Heterozygous.

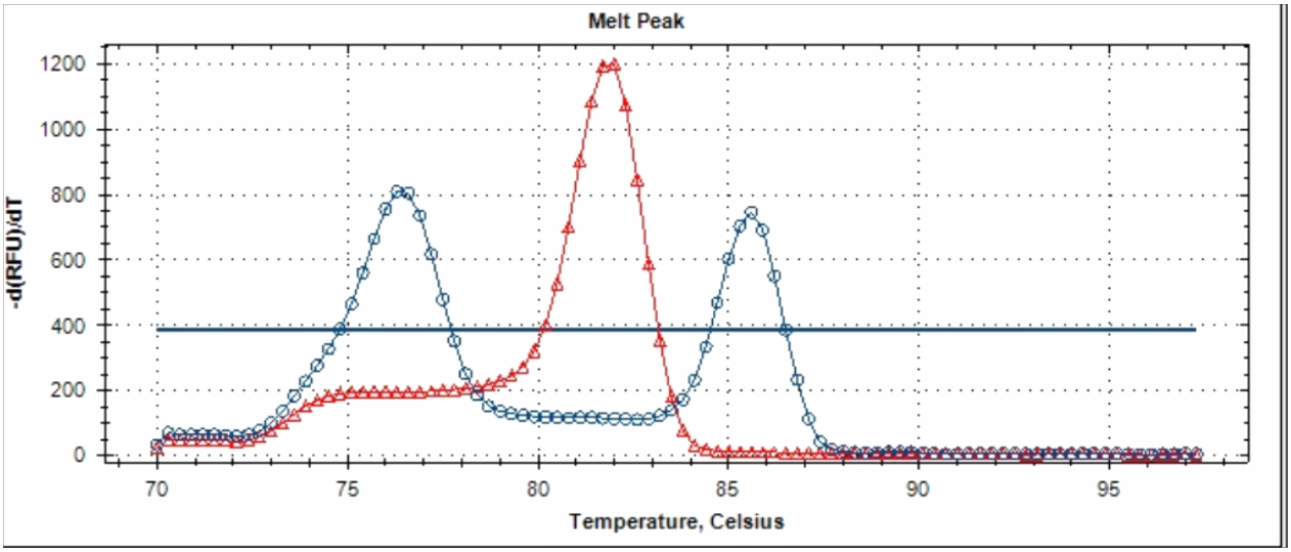


Mix3,4: V726A Heterozygous.

11.3 Homozygous patient results



Mix1,2: M694V Homozygous.



Mix3,4: M680I Homozygous.

12. Limitation of the kit

This kit can only detect the most frequent 5 mutations which indicates by this IFU, The kit can not detects other MEFV gene mutations.

13. References

1. Booty et al. Familial Mediterranean fever with a single MEFV mutation: Where is the second hit?, Arthritis Rheum. 2009 June ; 60(6): 1851–1861. doi:10.1002/art.24569

14. Notes

This kit has CE-IVD approved and validated for human in vitro diagnostics.

15. Revision History

Date of Last Edit: December 2024		
Change	**Affected Section**	**Page**
Added reaction numbers for the kit	4.1 Reagents	5
Added ∑ (total numbers of tests) symbol	Cover Page	1
Added optiamal concentrations for DNA sample	4.1 Reagents	5
Added Safety Instructions and General Warnings	2. Safety Instructions and General Warnings	3
Format updated	3.1 Package Contents	4

16. Troubleshooting

geneMAP™ FMF 5 Mutation Detection Kit		
OBSERVATION	**POSSIBLE CAUSES**	**SOLUTION**
No signal in fluorophore	Fluorophores incompatible with protocol for data analysis	Select the correct fluorophores.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Repeat the test with the new extracted nucleic acid.
Melting curve signals in Negative Control	Cross Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips and throughout the procedure and change tips between tubes. Repeat entire procedure from nucleic acid extraction with the new set of reagents.
No melting curve signal in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please carefully repeat the test.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 4) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.

17. Symbols Used

REF	Catalog Number
LOT	Lot/Batch Number
	Expiration Date
-15°C -25°C	Storage Conditions
	Manufactured by
CE IVD	Intended Use

18. Contact Information

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