Advanced Molecular Diagnostics Ltd Zena Max Familial Mediterranean Fever (FMF) PCR Kit

CE IVD

REF **KD6466292-100**

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

This kit contains an *in vitro* PCR assay for the qualitative determination of Familial Mediterranean Fever MEFV polymorphisms in human samples, such as whole blood and EDTA plasma, based on (FMF) TaqMan® detection method for MEFV polymorphisms associated with FMF, with a highly sensitive qPCR.

For *in vitro* diagnostic use only.

Overview

Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disorder caused by pathogenic variants in the MEFV gene, which encodes the protein pyrin. Pyrin plays a key role in the regulation of the innate immune response, particularly in the activation of the inflammasome and interleukin-1β (IL-1β) production. Mutations in MEFV lead to inappropriate or prolonged inflammatory signalling, resulting in recurrent episodes of fever, serositis, and elevated acute-phase reactants. FMF predominantly affects individuals of Mediterranean descent, including populations from the Middle East, North Africa, and southern Europe. Although disease severity varies depending on the specific mutation, recurrent attacks of peritonitis, pleuritis, arthritis, or erysipelas-like rash are characteristic features. Amyloidosis (AA type) remains a serious long-term complication, especially in untreated individuals.

Diagnosis is supported by clinical presentation, elevated inflammatory markers during attacks, and genetic testing for MEFV mutations. Colchicine is the standard therapy, effectively preventing attacks and amyloidosis in most patients, while IL-1 inhibitors may be used in colchicine-resistant cases. Early detection and genotype-informed management are critical for reducing morbidity and improving quality of life in FMF patients.

Principle of the Test

The qPCR kit is designed for the detection of FMF polymorphisms by the real-time Polymerase Chain Reaction (PCR) method detection is based on the amplification of a specific conserved DNA sequences and measuring the amplification product concentration using PCR process and fluorophore labelled probes. For the DNA isolation quality control and possible PCR inhibition control there are primers and probe for internal control gene amplification present in the reaction mix. Amplification of internal control gene is indicated in the Cy5 fluorophore fluorescence channel. The detection kit utilizes the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. The ready-to-use master mix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by

amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

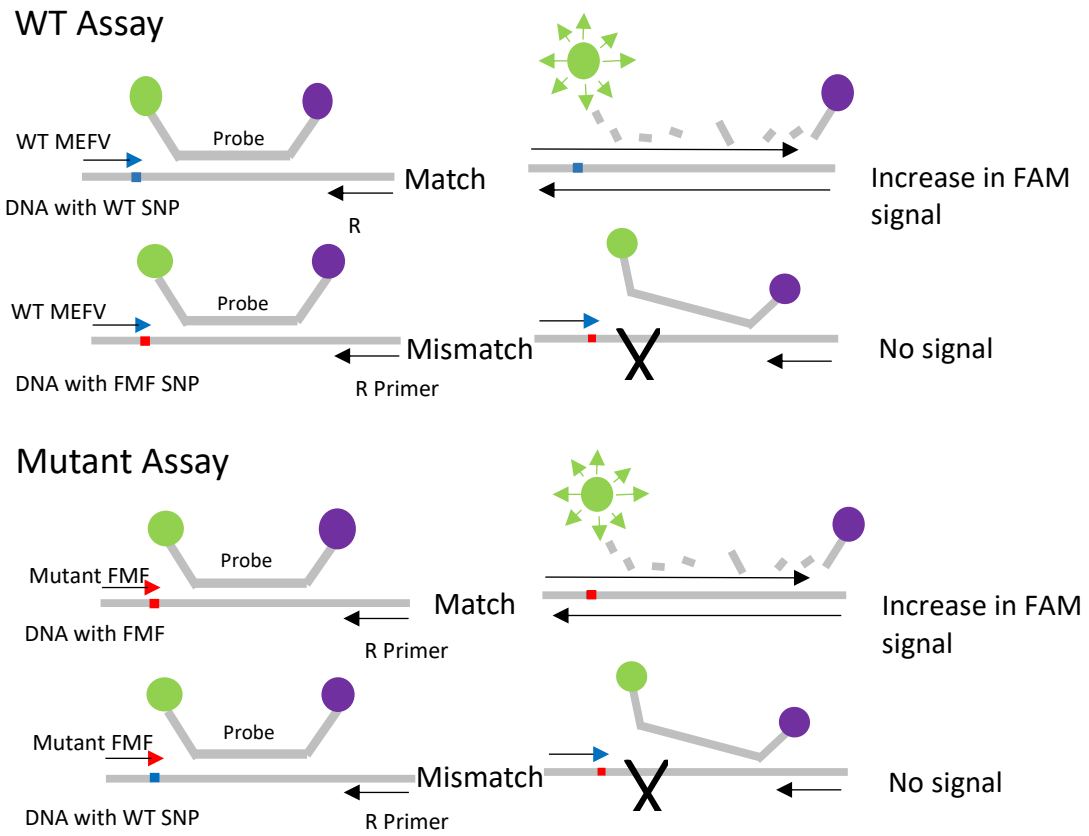

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of FMF. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. The FMF assay will produce a FAM signal if FMF is present; however, if it is not present, no FAM signal will be detected. Due to assay competition, the HEX signal may be reduced or absent when the FAM signal is strong.

Materials Provided

Kit Contents

Product	Volume
Master Mix 1a (WT M694, E148, V726)	1 x 1.0 ml
Master Mix 1b (Mut M694V, E148Q, Y726A)	1 x 1.0 ml
Master Mix 2a (WT M694, R761, M680)	1 x 1.0 ml
Master Mix 2b (Mut M694I, R761H, M680I)	1 x 1.0 ml
Master Mix 3a (WT P369, A744, A479)	1 x 1.0 ml
Master Mix 3b (Mut P369S, A744S, A479L)	1 x 1.0 ml
Master Mix 4a (WT M695, M1105, R653)	1 x 1.0 ml
Master Mix 4b (Mut M695R, M1105L, R653H)	1 x 1.0 ml
FMF DNA control	1 x 0.05 ml
WT DNA control	1 x 0.05 ml
Nuclease-free water	1 x 1 ml

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -18°C and -25°C. The kit will remain stable at least until the expiry date printed on the package if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

Materials and Equipment Required (but not provided)

DNA Extraction: AMD manufactures the LUCO Blood DNA Extraction Kit (KD474881-50), which can be used for extracting DNA from the samples. Other leading brands of IVD extraction kits are acceptable for use with this diagnostic kit.

PCR Instrument: AMD have validated the use of this kit on most major qPCR machines and recommends for use with this PCR kit.

Consumables: AMD manufactures a high-quality nuclease and pyrogen-free PCR plasticware suitable for use with this kit. Use of other manufacturers' consumables is also acceptable.

Reagents: Nuclease-free water

Other Laboratory Equipment: Vortex, micro centrifuge, micro pipettes and tips, microfuge tube rack, PCR tube/plate rack, spectrophotometer.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Discard sample and assay waste according to your local safety regulations. It is essential to follow the instructions in this manual precisely to ensure the best product performance.

Sample Collection, Storage and Transport

Whole blood can be taken into EDTA tubes and stored for a maximum of 6 hours at 2-8^{0}C before sample preparation. Aliquot and store the samples at -20^{0}C to -80^{0}C immediately if they are not to be used within this time period. Freeze-thawing may compromise the test results. Ensure that samples are stored correctly and kept away from any contamination.

For transportation, the samples should be placed in a shatterproof transport container to avoid the potential danger of infection due to sample leakage. Transport samples following the local and national instructions for the transport of pathogenic material, by courier, if possible, at a temperature of 2-8^{0}C and no longer than 6 hours following collection.

Assay Procedure

Sample Preparation

For optimal results, use the LUCO AMD DNA Extraction Kit (KD474881-50) to extract the DNA from the sample. It is important to ensure that all samples are kept free from any contamination and that correct storage procedures are followed to ensure there is no damage

to the DNA. Store the DNA at 2-8°C for up to 24 hours, then at -20°C for longer-term storage to ensure there is no damage to the DNA.

PCR Set up

1. Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
2. Set up the reactions on a cool block or ice using the table below, ensuring to include duplicate reactions for all samples and controls. If preferred, make a mix of assay and reverse enzyme mix and aliquot 20µL into the PCR tubes/plate.

Product	Volume
FMF polymorphisms Master Mix	20 µl
DNA Sample/Control	5 µl
Total	*25 µl*

3. Add the DNA samples and standards to the PCR tubes/plate. Also, add 5 µl nuclease-free water in place of the DNA as a No Template Control (NTC).
4. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present.
5. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile

Stage/Step	Temperature	Time
Stage 1: Step 1	40˚C	5mins
Stage 1: Step 2	95˚C	5mins
35 Cycles		
Stage 2: Step 1	95˚C	10secs
Stage 2: Step 2*	60˚C	30secs

*Data collection step in FAM, HEX, ROX, and Cy5 channels.

6. When setting up the sample information in the qPCR software, enter the concentration of the FMF standards and define them as standards in order to automatically obtain a standard curve and quantification of the amount of FMF which may be present in the sample.
7. When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

8. Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Master Mixes	A (1/2/3/4)			B (1/2/3/4)			IC	Interpretation
	FAM	HEX	ROX	FAM	HEX	ROX	Cy5	
	+	+	+	-	-	-	+	Negative FMF
	-	-	-	+	+	+	+	Positive FMF
	+	+	+	+	+	+	+	Heterozygous carrier
	-/+	-/+	-/+	-/+	-/+	-/+	-	Inconclusive

Table 1: Basic Ct Interpretation guidance.

*It should be noted that if any of the target parameters in **Master Mix B** show amplification (Ct detected), the reaction should be interpreted as **positive for Master Mix B**, even if not all parameters are present. Heterozygous individuals typically present with milder or carrier-like symptoms, since FMF is usually autosomal recessive; however, there are documented cases with dominant inheritance or symptomatic heterozygosity. A detailed guidance table is available upon request.*

Interpretation of Results

This is a qualitative assay, and the results are scored based on whether Cq values are present or not. Results should be interpreted as follows, using Table 1 as a quick reference guide:

- The internal control assay signal in the Cy5 channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay signal is strong.
- If there is a signal in the FAM (green) channel, with or without a WT signal, the sample is **heterozygous** for FMF.
- If there is a HEX, ROX or FAM signal but no WT signal, the sample is **positive** for FMF.
- If there is a WT signal but no HEX, ROX or FAM, then the sample is **negative** for FMF.
- If there is no signal in either channel, the result is **inconclusive**.

Product Limitations

This kit is for *in vitro* diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally, mutations may arise in the genomic region targeted by the primers and probes of this assay, leading to a reduction in performance or failure of the assay. The assay design and efficacy are reviewed periodically. Rare novel mutations outside the assay's target regions may not be detected.

This test is intended as a supportive diagnostic tool and should be interpreted alongside clinical history and standard FMF diagnostic criteria. Results should not be used as the sole basis for diagnosis or treatment.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA, and population genotyping studies, to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity. Please familiarise yourself with the qPCR instrument before using the AMD FMF qPCR Kit.

Contact

If you have any queries, comments or complaints please refer to our website at:

www.am-diagnostics.co.uk

info@am-diagnostics.co.uk

References:

1. Al-Wahadneh AM, Dahabreh MM. Familial Mediterranean fever in children: a single centre experience in Jordan. *Eastern Mediterranean Health Journal.* 2006;12(6):818-823.
2. National Organisation for Rare Disorders. Familial Mediterranean Fever (FMF). Accessed 06.11.2025. Available: https://rarediseases.org/rare-diseases/familial-mediterranean-fever/
3. Amr SS. Familial Mediterranean fever among Arabs. Annals of Saudi Medicine. 1989;9(6):519-524.

Harmonised Symbols

The following is a key of all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	UDI-DI number for the product given	Basic: 506105998FMFGN UDI-DI: (01)05061059980281 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD6466292-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC