Manual

MutaPLATE® Cystic Fibrosis

Real-Time-PCR-Kit

For the analysis of the F508del mutation in the Cystic Fibrosis Transmembrane Conductance Regulator Gene (CFTR)

Valid from 2022-06-15

REF **KF190832**
KF190896

Σ 32/96

-20 °C

IVD

CE

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1 INTENDED USE

The MutaPLATE® Cystic Fibrosis Real-Time PCR Kit is a molecular biology assay based on TaqMan technology for the examination of the F508del mutation in the Cystic Fibrosis Transmembrane Conductance Regulator Gene (CFTR) from genomic DNA. The examined variation is connected to the development of severe symptoms of Cystic Fibrosis (Mucoviscidosis).

2 INTRODUCTION

Cystic fibrosis is a disease caused by a malfunction of the secretory epithelium of all excretory glands. It is based on genetic defects in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene and is inherited on in an autosomal-recessive fashion. It is the most common congenital metabolic disorder in the Caucasian population. The incidence of cystic fibrosis among Caucasians in very high: 1 in 2500 newborns is affected by the disease.

The consequence of the genetic defects is a disruption in the electrolyte transport. A protein responsible for the chloride permeability in a cell is either non-functional or not translated at all or it cannot be transported to the cell membrane. Consequently the exocrine glands secrete a highly viscous fluid that cannot drained off the glands leading to severe inflammations as a consequence. These factors result in the syndromic clinical appearance with the main characteristics being a chronic obstructive pulmonary disease and exocrine pancreas insufficiency.

A genetic examination of the CFTR gene in newborns can provide an early diagnosis and a prompt therapy which can greatly enhance life expectancy as well as quality of life. [1], [2], [3], [4], [5]

3 PRINCIPLE OF THE TEST

The MutaPLATE® Cystic Fibrosis Real-Time PCR Kit contains two specific primers that flank the target sequence and two hydrolysis probes (TaqMan probes) that bind specifically in the region of the mutation. The two hydrolysis probes are labelled at the 5' end with different fluorophores (reporter dyes), which are used to distinguish the alleles. At the 3' end, the probes are labelled with a non-fluorescent quencher. The proximity of the reporter dye to the quencher inhibits the fluorescence of the reporter molecule. During amplification, the probes bind specifically to the DNA fragments. The 5' nuclease activity of the polymerase cleaves the hybridised probes, separating the reporter from the quencher and generating a fluorescent signal.

4 PACKAGE CONTENTS

The components supplied are sufficient for the preparation of 32 (KF190832) or 96 (KF190896) reactions.

Table 1: Components of the MutaPLATE® Cystic Fibrosis Real-Time-PCR Kit .

Label	Lid Colour	Content	
		32	96
Enzyme mix	blue	1 x 438 µl	3 x 438 µl
Detection mix	yellow	1 x 368 µl	3 x 368 µl
Positive control	red	1 x 45 µl	1 x 45 µl
Negative control	green	1 x 150 µl	1 x 150 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA extraction kit (e.g. MutaCLEAN® Universal RNA/DNA, KG1038)
- Open Real-Time PCR Instrument
- Cooling block for PCR reaction tubes
- Sterile reaction tubes
- Calibrated pipettes (variable volumes) and sterile disposable tips with filter
- Optional: Liquid handling system for automation

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLATE® Cystic Fibrosis real-time PCR kit is transported frozen on dry ice or cold packs. All components are to be stored protected from light at a minimum of -20 °C immediately after receipt. Avoid more than three freeze-thaw cycles of the detection mixes (make aliqouts if necessary). The positive and negative control and the detection mix can be stored at 4 – 8 °C after initial thawing for up to three months. Do not use after the expiry date indicated on the package.

Be sure to protect the detection mixes from direct sunlight during the entire test period

7 WARNINGS AND PRECAUTIONS

Read the instructions for use carefully before using the product.

- All samples must be considered potentially infectious and/or biohazardous and all items that come into contact with the specimens must be considered potentially contaminated.

- Real-time PCR must be performed in laboratories suitable for this purpose and by specially trained personnel.
- The assay must always be carried out according to the instructions supplied with the kit.
- Areas for sample preparation and preparation of the PCR master mix should be strictly separated.
- Pipettes, tubes and other working materials must not circulate from one area to the other.
- Always use pipette tips with filters.
- Always wear powder-free disposable gloves when using the kit
- Clean pipettes and work surfaces regularly with suitable decontamination solution (no ethanol-containing agents).
- Contamination of eluates and kit components with microbes or nucleases (RNAs and DNAses) should be avoided.
- Positive and potentially positive material must be kept separate from all other kit components at all times.
- Do not open reaction tubes/plates after amplification in order to avoid contamination.
- In accordance with guidelines or requirements of local, state or federal regulations or authorised organisations, additional controls may be tested.
- Do not autoclave reaction tubes after PCR as this will not degrade the amplified nucleic acid and risks contaminating the laboratory area.
- Dispose of samples and test waste according to your local safety regulations.
- Refrigerate all PCR reagents while working.
- The purity (A260/A280) of the genomic DNA should be between 1.8 and 2.0.

8 SAMPLE MATERIAL

Starting material for the MutaPLATE® Cystic Fibrosis real-time PCR kit is genomic DNA isolated from clinical samples (blood) using a suitable extraction kit.

9 REAL-TIME-PCR

9.1 Important points before starting

- Please pay attention to chapter 7 "Warnings and precautions".
- Before setting up the Real-Time-PCR familiarise yourself with the Real-Time PCR instrument and read the user manual supplied with the instrument.

- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run one Positive Control and one Negative Control should be included.
- Before each use, all reagents must be gently thawed, thoroughly mixed (do not vortex) and briefly centrifuged.
- Protect the detection mixes from exposure to light.
- We recommend always cooling the reagents and the preparation in a cooling block (+4 to +8 °C) or on ice while working.

9.2 Procedure

For amplification, one reaction tube (Type depending on the device used) per sample and two additional reaction tubes for the negative and the positive control are required. The following table shows the volumes to be pipetted per sample. For the analysis it is recommended to prepare a master mix for the number of samples (incl. negative and positive control) (N) plus 10 % to compensate for inaccuracies. The master mix is pipetted as described in Table 2:

Tabel 2: Preparation of master mix

Reagent	Volume per 25 µl - reaction mix	Master mix volume
Detection mix (**yellow**)	10.5 µl	10.5 µl * (N + (N * 0.1))
Enzyme mix (**blue**)	12.5 µl	12.5 µl * (N + (N * 0.1))

- Mix the Master Mix carefully by pipetting up and down or by inverting and centrifuge briefly.
- Add **23 µl** of the Master Mix to each reaction tube.
- For the negative control add **2 µl** of the supplied negative control (**green**).
- For the positive control add **2 µl** of the supplied positive control (**red**).
- For each sample to be analysed, add **2 µl** of the extracted genomic DNA to the corresponding reation tube.

Close the reaction tubes and centrifuge. Then transfer to the real-time PCR device and start the PCR programme described in 9.3.

9.3 Instrument settings

For the Real-Time-PCR use the thermal profile shown in table 3.

Table 3 Real-Time-PCR thermal profile

Description	Time	Temperature	Heating rate	Cycles	Acquisition
Initial Denaturation	120 s	94 °C	max.	1	none
Denaturation	10 s	94 °C	max.	40	none
Primer annealing and Elongation	60 s	56 °C	max.		**single**
Cooling	30 s	40 °C	max.	1	-

10 DATA ANALYSIS

The MutaPLATE Cystic Fibrosis real time PCR Kit contains specific primers and additional material for the detection of the F508del mutation in the Cystic Fibrosis Transmembrane Conductance Regulator Gene (CFTR) from genomic DNA.

The standard PCR contains additionally **two sequence specific oligonucleotides** marked with fluorescence dye (TaqMan probes). Both probes bind at the amplificated target-DNA which includes the F508del mutation. Due to this, a fluorescence signal is generated and detected by the optical unit of the used real time PCR instrument. The TaqMan probe for the wildtype is marked with FAM (**510 nm, green**) and the TaqMan probe for the F580del is marked with YAK (**555 nm, yellow**).

The Colour Compensation Kit MutaPLATE® CC-1 (KF19-3-CC) is required for the analysis on Roche LightCycler® devices.

According to the genotype, the following three discriminations can be obtained:

1. homozygous wild type:

Increase in fluorescence signal from **FAM** labelled TaqMan probe and no increase in fluorescence signal from **YAK** labelled TaqMan probe.

2. heterozygous mutation:

Increase in fluorescence signal from the **FAM** labelled TaqMan probe and increase in fluorescence signal from the **YAK** labelled TaqMan probe.

3. homozygous mutation:

No increase in fluorescence signal from the **FAM** labelled TaqMan probe and increase in fluorescence signal from the **YAK** labelled TaqMan probe.

The following figure shows representative results for experiment: **black and pink curve** - negative control, **green curve** - heterozygous wildtype / F508del mutation.

FAM (510 - 530 nm, green) - presence of wild type gene

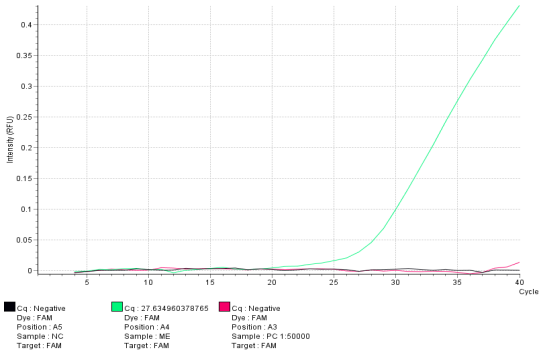

Fig. 1: Evaluation **FAM** (**510 - 530 nm, green**) - presence of wildtype gene

YAK (550 - 570 nm, yellow) - presence of the F508del mutation

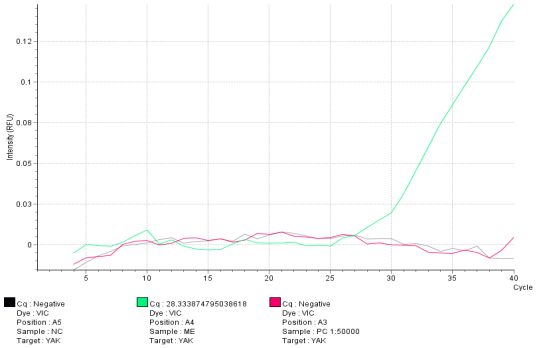

Fig. 2: Evaluation **YAK (550 - 570 nm, yellow)** - presence of the F508del mutation

The supplied Positive Control (**red**) contains a template that is heterozygous for the F508del mutation.

11 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time-PCR. If you have further questions, please do not hesitate to contact our scientists on info@immundiagnostik.com.

No or weak fluorescence in the positive control or samples.

Check the PCR programme of the real-time PCR system and repeat the analysis with the corrected protocol.

The Detection Mix has been subjected to more than three freeze cycles or have been stored at 2-8 °C for more than three month. Repeat the analysis with a fresh aliquot or a new detection mix.

The quality of the starting DNA is not sufficient. Use freshly extracted DNA and determine the concentration/purity before use.

The detection mixes were not protected from light exposure. Repeat the analysis with a fresh aliquot or new PCR reagents.

12 KIT PERFORMANCE

12.1 Analytical Sensitivity

The limit of detection (LoD) of the MutaPLATE® Cystic Fibrosis Real-Time PCR kit was determined using serial dilutions of synthetic DNA-fragments containing the F508del mutation target sequence in a Roche LightCycler® 2.0 real time PCR instrument. The LoD of the MutaPLATE® Cystic Fibrosis Real-Time PCR kit is 1000 genome copies per reaction each.

12.2 Precision

The precision of the MutaPLATE® Cystic Fibrosis Real-Time PCR kit was determined as intra-assay variability and inter-assay variability.

Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of the F508del mutation target sequence.

Wild type (FAM)	**copies/ µl**	**Standard deviation**	**Coefficient of variation [%]**
Intra-Assay Variability	3000	1.17	1.38
Inter-Assay Variability	3000	1.27	1.61

Mutation (YAK)	**copies/ µl**	**Standard deviation**	**Coefficient of variation [%]**
Intra-Assay Variability	3000	0.70	0.49
Inter-Assay Variability	3000	2.28	5.22

12.3 Diagnostic Senstivity

The diagnostic sensitivity of Real-Time-PCR assays is mainly dependent on the DNA extraction method used to isolate DNA from various biological specimens. DNA extraction reagents are not part of the Immundiagnostik AG Real-Time-PCR kits. Immundiagnostik AG Real-Time-PCR kits include an positive and negative control. Therefore, Immundiagnostik AG guarantees the analytical sensitivities and specificities of the Real-Time PCR kits, performed with eluted DNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. Immundiagnostik AG does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of Immundiagnostik AG Real-Time-PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods. It is the responsibility of the user to qualify the extraction methods used for DNA isolation from biological samples.

13 LIMITATIONS OF THE METHOD

The accuracy of genetic testing is never 100%. However, an accuracy of more than 98% was determined based on the validation data. The attending physician is free to use the test results as a guidance in the decision making process in terms of diagnosis and therapy. However, these recommendations are based on genetic test outcomes and need to be interpreted in the context of medical history and known familiar risks of each individual patient. The attending physician is fully responsible for the final diagnosis and treatment.

The test only analyzes a selection of markers. For the detection of alleles the examined polymorphisms are indicated. Other rare alleles might be present, which are not covered by this method. Thus a negative test result does not exclude a risk for the patient in any form.

14 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleic acid	REF	Catalog number
PCR	Polymerase chain reaction	→REF	To be used with
ENZYME MIX	Enzyme mix	Σ	Contains sufficient for <n> test

DETECTION MIX	Detection mix		Upper limit of temperature
CONTROL +	Positive control	CONTENT	Content
CONTROL –	Negative control		Manufacturer
IVD	*In vitro* diagnostic medical device	LOT	Lot number
	Use by YYYY-MM-DD		Consult instructions for use

15 LITERATURE

1. G. Dockter et al., Mukoviszidose. Zystische Fibrose 1992
2. N. Dray-Charier et al.,Hepatology, 29(6):1624–1634, Jun 1999
3. L. Frentescu et al., J Cyst Fibros, 7(5):423–428, Sep 2008
4. A. Vankeerberghen et al., J Cyst Fibros, 1(1):13–29, Mar 2002
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