Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

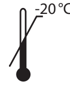
MutaPLATE® Laktase (TM)

Real-Time-PCR Kit

For the analysis of the T-13910C mutation in the LCT gene

Valid from 2022-03-15

KF1907232
KF1907296



Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1 **INTENDED USE** 13
2 **INTRODUCTION** 13
3 **PRINCIPLE OF THE TEST** 13
4 **PACKAGE CONTENTS** 13
5 **EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER** 14
6 **TRANSPORT, STORAGE AND STABILITY** 14
7 **WARNINGS AND PRECAUTIONS** 14
8 **SAMPLE MATERIAL** 15
9 **REAL-TIME-PCR** 15
9.1 Important points before starting 15
9.2 Procedure 15
9.3 Instrument settings 16
10 **DATA ANALYSIS** 16
11 **TROUBLESHOOTING** 18
12 **LIMITATIONS OF THE METHOD** 18
13 **ABBREVIATIONS AND SYMBOLS** 19
14 **LITERATURE** 19

1 INTENDED USE

The MutaPLATE® Laktase (TM) Real-Time PCR Kit is a molecular biology assay based on TaqMan technology for testing the T-13910C point mutation in the LCT gene.

2 INTRODUCTION

Lactose is a form of sugar found in milk. In order for lactose to be absorbed, it must be broken down by the enzyme lactase. Variations in the lactase (LCT) gene can lead to an impairment of the enzyme activity, whereby lactose is not or only partially split and therefore cannot be absorbed. Lactose remaining in the intestine is processed by bacteria into lactic acid. This can lead to the symptoms typical of lactose intolerance, such as abdominal cramps, nausea and diarrhoea. [1]

3 PRINCIPLE OF THE TEST

The MutaPLATE® Laktase (TM) 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 (KF1907232) or 96 (KF1907296) reactions.

Table 1: Components of the MutaPLATE® Laktase (TM) 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 15 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® Laktase (TM) 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 multiple freeze-thaw cycles (make aliqouts if necessary). 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 surfLaktases 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® Laktase (TM) 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 plLaktase 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	30 s	94 °C	max.	45	none
Primer annealing and Elongation	60 s	66 °C	max.		**single**
Cooling	30 s	40 °C	max.	1	-

10 DATA ANALYSIS

The TaqMan probe for the C allele (mutation) is marked with **FAM** (**510 - 530 nm, green**) and the TaqMan probe for the T allele (wild type) is marked with **YAK** (**550 - 570 nm, yellow**).

According to the genotype, the following results can be obtained:

1. homozygous mutation:

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 wild type:

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

The evaluation of the amplification curves (determination of the crossing points) is carried out with an analysis of the type „absolute quantification". The result of the mutation allele is analysed at **510 - 530 nm / green** and for the result of the wild-type allele at **550 - 570 nm / yellow**. **The Colour Compensation Kit MutaPLATE® CC-1 (KF19-2-CC) is required for the analysis on Roche LightCycler® devices.**

Analysis LCT T-13910C

The following graphs show the typical results for samples with the genotypes homozygous wild type, heterozygous mutated and homozygous mutated: **blue curve** - negative control, **green curve** - homozygous wild type, **red curve** - heterozygous mutated, **black curve** - homozygous mutated.

FAM (510 - 530 nm, green) - detection of the C allele (mutation)

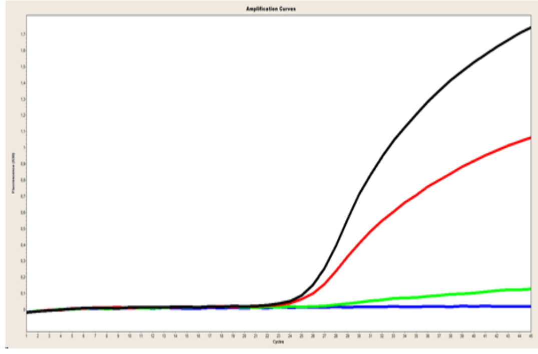


Fig. 1: Evaluation **FAM (510 - 530 nm, green)** - detection of the C allele (mutation).

YAK (550 - 570 nm, yellow) - detection of the T allele (wild type)

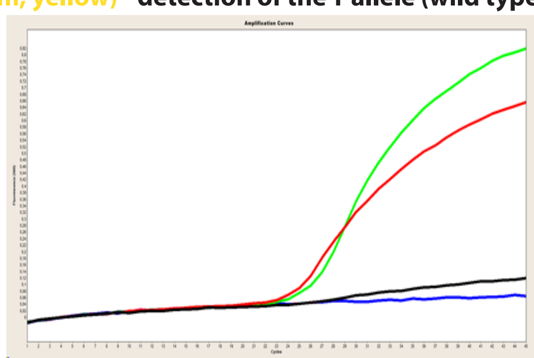


Fig. 2: Evaluation YAK (550 - 570 nm, yellow) - detection of the T allele (wild typ).

The supplied Positive Control (**red**) contains a template that is heterozygous for the point mutation T-13910C.

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 two freeze cycles or have been stored at 2-8 °C for more than four days. 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.

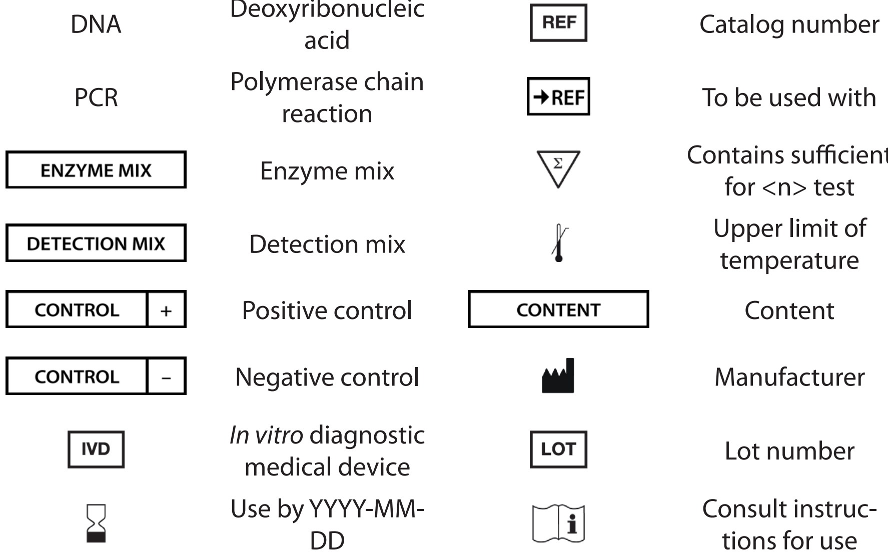
12 LIMITATIONS OF THE METHOD

The result is provided to the treating physician as supportive material and should never be used and should never be used solely for diagnostic or treatment recommendations for treatment. The diagnosis and the treatment decisions remain the full responsibility of the physician.

The accuracy of genetic tests is not 100 %. However, an accuracy of over 98 % based on validation data for this test. Furthermore, results of genetic tests must be considered in the context of the clinical representation of the patient and known familial risks in the environment of the patient.

The test only analyses a selection of markers. When alleles are detected, the investigated polymorphism is indicated. Other rare alleles may be present and are not covered by this method. Therefore, a negative test result in a patient of the patient does not completely exclude a risk of any kind.

13 ABBREVIATIONS AND SYMBOLS



Abbreviation / Symbol	Meaning	Symbol	Meaning
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	i	Consult instructions for use

14 LITERATURE

1. Mattar et al., Clinical and Experimental Gastroenterology 2012, 5:113-121