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MutaREAL® Laktase

Real-Time-PCR Kit

For the analysis of the insertion/deletion polymorphism in the Laktase gene

Valid from 2021-09-07

REF **KF2907132**
KF2907196

Σ 32/96

-20 °C

IVD

CE

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1 INTENDED USE

The MutaREAL® Laktase Real-Time PCR Kit is a FRET-based molecular biology assay 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 sequence-specific MutaREAL® Laktase real-time PCR kit is based on fluorescence resonance energy transfer (FRET).

The assay contains two specific primers flanking the target sequence and two hybridisation probes that bind adjLaktasent to the target sequence. One of the hybridisation probes is labelled with a donor fluorophore and, after appropriate excitation, transfers its energy to the acceptor fluorophore with which the other hybridisation probe is labelled when they are in close proximity. After the energy transfer, the acceptor dye emits light with a longer wavelength. Energy transfer can only occur when both hybridisation probes have bound to the target sequence. The amount of hybridised probe pairs and thus the fluorescence signal increases with the amount of amplified PCR product. Here, the fluorescence signal is proportional to the amount of PCR product.

Genotyping is carried out after completion of amplification by melting curve analysis. For this purpose, the temperature is slowly increased after a denaturation step and the dissociation behaviour of the hybridisation probes is recorded while continuously measuring the fluorescence. One of the hybridisation probes binds to a part of the target sequence that is present in the wild type and the mutation. The second hybridisation probe spans the mutation site. As the temperature rises, the mismatched and thus less stable probes dissociate first and the fluorescence decreases. The perfectly paired hybridisation probes dissociate later due to their higher binding energy and thus the fluorescence signal decreases only at a higher temperature.

4 PACKAGE CONTENTS

The components supplied are sufficient for the preparation of 32 (KF2907132) or 96 (KF2907196) reactions.

Table 1: Components of the MutaREAL® Laktase 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 50 µl	1 x 100 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA extraction kit (e.g. MutaCLEAN® Universal RNA/DNA, KG1038)
  (the CE conformity only exists if one of the mentioned devices is used)
- Roche LightCycler® 1.5, 2.0 or 480 real-time PCR system
- Roche LightCycler® capillaries resp. 96-well plates/stripes (white)
- Roche LightCycler® Cooling Block
- 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 MutaREAL® Laktase 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 MutaREAL® Laktase 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 capillary.
- 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 capillary.

LightCycler® 1.5 and 2.0: Close the capillaries with the lids, transfer them into the LightCycler® carousel and centrifuge them in the LightCycler® centrifuge (if a table-top centrifuge is used, centrifuge the capillaries in the inserts of the cooling block at 3000 rpm for 15 s). Then transfer the carousel to the LightCycler® and start the PCR programme described in 9.3.

LightCycler® 480: Seal the wells with a sealing foil and centrifuge the plate (2000 rpm for 15 s). Then transfer the plate into the LC 480 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.	50	none
Primer annealing	25 s	55 °C	max.		**single**
Elongation	25 s	72 °C	max.		none
Melting curve	20 s	94 °C	max.	1	none
	20 s	40 °C	max.	1	none
	0 s	80 °C	0.1 - 0.2 °C/s*	1	**constant**
Cooling	30 s	40 °C	max.	1	-

*Depending on the number of filters selected for the LightCycler® 480, it may be necessary to adjust the acquisitions per °C.

10 DATA ANALYSIS

Add an analysis of the type „genotyping“ for the evaluation of the melting curves. This forms the derivative of the fluorescence curve. The detection wavelength is between 660 nm and 705 nm (corresponding to the real-time PCR device used).

Temperature T-allele: 50.0 °C (+/-2 °C)

Temperature C-allele: 60.0 °C (+/-2 °C)

The following graph shows the typical results for the possible genotypes: **light blue curve** - negative control, **green curve** - homozygous TT, **red curve** - heterozygous TC, **blue curve** - homozygous CC.

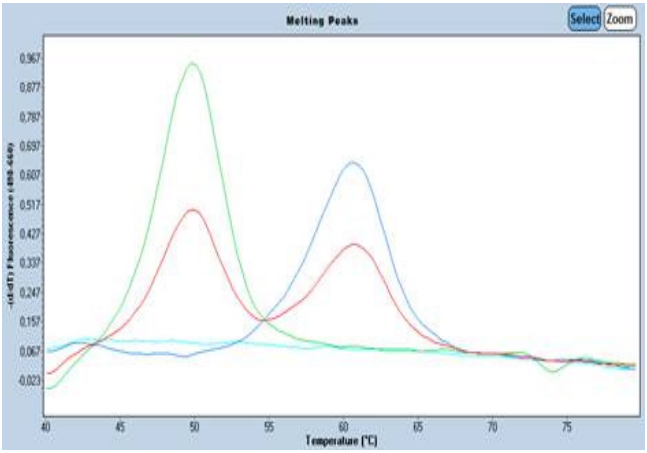

Fig. 1: Evaluation of melting curve

The positive control provided contains a template that is heterozygous for the point mutation T- 13910C is heterozygous.

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

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		Manufacturer
CONTROL –	Negative control	LOT	Lot number
IVD	*In vitro* diagnostic medical device		Consult instructions for use
	Use by YYYY-MM-DD	CONTENT	Content

14 LITERATURE

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