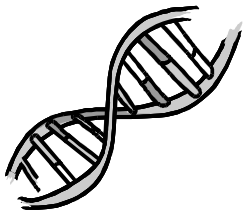
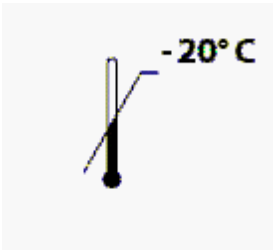


MutaPLATE® Laktase (TM)

(TAQ-Man) real time PCR Kit

PCR test for analysis of -13910 T/C polymorphism in the regulatory region of the lactase phlorizin hydrolase (LPH) gene (genetic lactose intolerance due to primary lactase deficiency) in open real time PCR systems (z. B. RotorGene, SmartCycler, Light Cycler, ABI, Amplifa, Stratagene) by Taq-Man technology.



IVD

for in vitro diagnostic only

CE

REF KF1907232 Σ 32

REF KF1907296 Σ 96

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
www.immundiagnostik.com Tel.: +49 (0)6251/ 701900
info@immundiagnostik.com Fax: +49 (0)6251/ 849430

Content

1 Intended Use

MutaPLATE ® Laktase (TAQ-Man) *real time* PCR kit is a molecular biological test for analysis of the -13910 T/C polymorphism in the regulatory region of lactase phlorizin hydrolase gene (LPH) in open real time PCR systems (e. g. RotorGene, SmartCycler, Light Cycler, ABI, Stratagene, Amplifa). The **clinical relevant C/C -** genotype causes primary lactase deficiency often leading to (genetic) lactose intolerance.

2 Introduction

Patients with **lactose intolerance** can not digest milk sugar and suffer after ingestion of milk-products from **dyspepsia, nausia and bellyache**. Further symptoms like vertigo, sleep disorders, akne or depressions can also be triggered by lactose intolerance. A Therapy for affected persons is very simple and can be done by **lactose-free diet**. In Germany, about 15 million people are affected from primary lactase deficiency [1].
The main reason for lactose intolerance is a **genetically based deficiency** of the enzyme lactase phlorizin hydrolase (LPH), which is responsible for the disassembly of milk sugar. This widely distributed genetic disorder is a T/C polymorphism located at position **–13910** in the regulatory region of this gene [2]. Person **homozygous for C/C**-genotype are consequently deficient for enzyme lactase and posses higher **risk for lactose intolerance**. These results are in excellent accordance with results obtained by the **lactose hydrogen breath test** for the diagnosis of lactase non-persistence [3]. Nevertheless, not all C/C-carriers must show typical symptoms because a fall short of individual level is necessary.
Furthermore, in some cases lactose intolerance can be due to **secondary** causes like mal-resorption problems (e. g. Morbus Crohn patients), infections or chemotherapy [4].
In **babyhood** and **infancy** the lactase production is very high but it decreases with **higher age** resulting in manifestation of primary lactase deficiency. Also a **North-/ South gradient** is visible: In Scandinavia the homozygous C/C-constellation is very rare whereas in Germany prevalence is about 15-20%. In Southern European countries up to 30% of all adults carry the C-allels homozygous.

Patients suffering from lactose intolerance have also a higher **risk for osteoporosis** due to the reduced calcium-intake via milk products [5]. In consequence, the C/C-genotype associated with primary lactose intolerance is a genetic risk factor for bone fractures for elderly people [6].

[1] Buning C, Ockenga J, Kruger S, Jurga J, Baier P, Dignass A, Vogel A, Strassburg C, Weltrich R, Genschel J, Lochs H, Schmitt H. (2003) The C/C (-13910) and G/G (-22018) genotypes for adult-type hypolactasia are not associated with IBD. Scand J Gastroenterol **38:** 538-542.
[2] Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. (2002). Identification of a variant associated with adult-type hypolactasia. Nat Genet **30:** 233-237.
[3] Högenauer C, Hammer HF, Mellitzer K, Renner W, Krejs GJ, Toplak H (2005). Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis fo lactase non-persistence. Eur J Gastroenterol Hepatol **17**: 371-376.
[4] Sibley E (2004) Genetic Variation/ Lactose Intolerance. Detection Methods/ Clinical Implications. Am J Pharmacogenomics **4** (4): 239-245.
[5] Obermeyer-Pietsch BM, Bonelli CM, Walter DE, Kuhn RJ, Fahrleitner-P A, Berghold A, Goessler W, Stepan V, Dobnig H, Leb G, Renner W (2004) Genetic predisposition for adult lactose intolerance and relation to diet, bone density, bone fractures. J Bone a. Mineral Research **19** (1): 42-47.
[6] Ennattah NS, Sulkava R, Halonen P, Kontula K, Järvelä I (2005) Genetic variant of lactase-persistent C/T- -13910 is associated with bone fractures in very old age. JAGS **53**: 79-82.

3 Principle of the test

MutaPLATE® Laktase (TAQ-Man) ***real time*** PCR Kit contains specific primers and additional material for the detection of the T/C (-13910) polymorphism in the regulatory region of the lactase phlorizin hydrolase gene. The variable area of the regulatory region from lactase gene is amplified by PCR using **genomic DNA-template**. The specific primers used in the kit flank the variable area of lactase gene (LCT) and generate an **amplificate of 300 bp** using Taq-Man technology.

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 single nucleotide polymorphism (SNP). 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 C-allele (mutation) is marked with **FAM (510 nm, green)** and the TaqMan probe for the T-allele (wildtype) is marked with **YAK (555 nm, yellow)**.

The following three discriminations are possible:

1. Homozygous **C/C**:
   Increase of the fluorescent signal from the **FAM** labeled TaqMan probe, no increase of the fluorescent signal from the YAK labeled TaqMan probe.
2. Heterozygous **C/T**:
   Increase of the fluorescent signal from the **FAM** labeled TaqMan probe and increase of the fluorescent signal from the **YAK** labeled TaqMan probe.
3. Homozygous **T/T**:
   No increase of the fluorescent signal from the FAM labeled TaqMan probe, increase of the fluorescent signal from the **YAK** labeled TaqMan probe.

4 Kit content

Each kit contains enough reagents to perform **32** respectively **96** tests. Each kit also contains a package insert.

Reference	Type of reagent	Volume (32x)	Volume (96x)
Blue	Enzymemix	435 µl	3 x 435 µl
Yellow	Detectionmix **T - Allele**	175 µl	3 x 175 µl
White	Detectionmix **C - Allele**	175 µl	3 x 175 µl
Red	Positive-Control	15 µl	3 x 15 µl
Green	Negative-Control	50 µl	3 x 50 µl

5 Required materials

Provided:

- Reagents for real-time PCR
- Package insert

Not provided:

- ***real time*** PCR capillary system (e. g. RotorGene)
- PCR reaction tubes
- Cryo container for PCR reaction tubes
- DNA extraktion kit for isolation of genomic DNA (ca. 10 ng/µl), e.g. **MutaCLEAN® DNA Blood, KG1033, Immundiagnostik**
- Pipetts (0,5 – 200 µl) with sterile filter Tipps for micro pipets
- sterile microtubes
- gloves (powder free)

6 Storage and handling

- All reagents should be **stored at <-20°C till immediate use**. Spin down kit components in their vials before long-term storage.
- **Avoid several freeze / thaw** cycles for the reagents (if necessary prepare suited aliquots and freeze again **immediately**).
- During preparation of PCR perform all working steps in a cryo-container (e.g. Light Cycler® Cooling block) or **cool all reagents** in suited manner.
- Primer-/ Probe-Mix should be **stored in the dark** (**light protection**).
- All reagents can be used until the expiration date (printed on the labels).

7 Warnings and precautions

- For ***in vitro*** diagnostic use only.
- This assay needs to be carried out by especially in molecular biology skilled personnel: This assay needs to be run according to GLP (Good Laboratory Practice).
- Clinical samples should be regarded as potentially infectious materials.
- Mix all reagents carefully before use, but do not vortex.
- Do not use the kit after its expiration date.

8 Test procedure

Before start, **decontaminate** all working areas and used instruments. Thaw kit components **gently at 8°C** and handle detection mixes in the dark. Prepare the necessary amount of PCR reaction tubes in a pre-cooled cooling block and consider additional 2 tubes for controls. Keep DNA samples ready and mix well before use.

Enzyme mix (***ready to use***)

This ready to use enzyme mix is stable for about 3 month at -20°C; after freezing, this solution can be thawed twice at 8°C provided that it was not stored longer than one hour (cooled) during the working steps.

Master mix preparation

Following table shows the composition for **one reaction** (final volume: 25 µl). For analysis of several samples in parallel, a **master mix** should be prepared in a sterile vial **multiplying** each single volume by the number **N** of samples (incl. controls). ***Additionally, 10% more volume should be calculated for reasons of inaccuracy***. The reagents should be pipetted in same order as indicated in the table:

Reagent	Volume	Master Mix Volume
Detection Mix (yellow)	**5 µl**	**5 µl** x (N + 10%)
Detection Mix (white)	**5 µl**	**5 µl** x (N + 10%)
Water (green)	**0,5 µl**	**0,5 µl** x (N + 10%)
Enzyme Mix *ready to use* (blue)	**12,5 µl**	**12,5 µl** x (N + 10%)

Mix prepared master mix well by gently pipetting (about **15 – 20 x, do not vortex**) and aliquot **23 µl** into each PCR reaction tube.

Samples

Add **2 µl** of each sample DNA in the corresponding PCR reaction tube; use first **both controls** (1. negative control, 2 µl and 2. positive control, 2 µl). Close the tubes and transfer them into the real time PCR instrument (keep position of samples).

Protocol

Activate following **PCR-protocol** and perform subsequently the ***real time*** PCR:

Experimental Protocol

Program:	**Denaturation**		1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Acquisition Mode
1	94	120	None

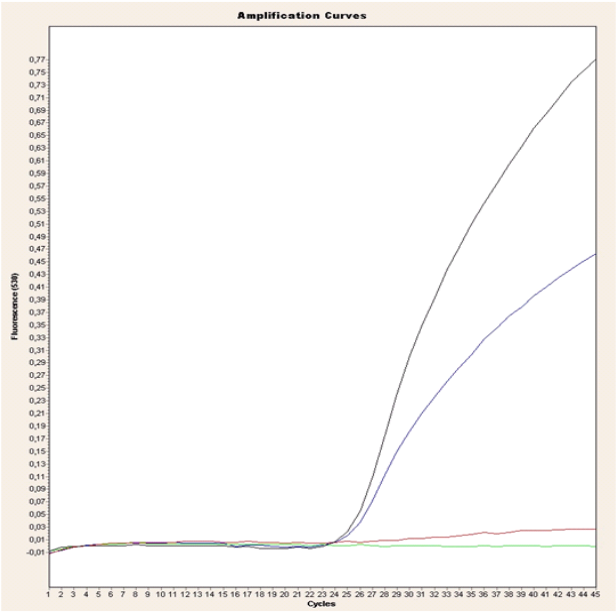
Program:	**Amplifikation**		45
Segment Number	Temperature Target (°C)	Hold Time (sec)	Acquisition Mode
1	94	30	None
2	65	60	**Single**
Program:	**Cooling**		1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Acquisition Mode
1	40	30	None

9 Analysis of genotype and interpretation of results

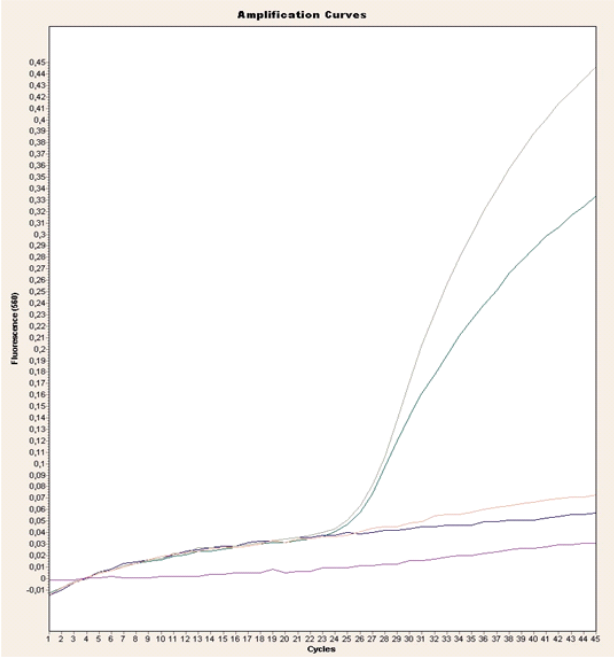
Results of the analysis for the T/C (-13910) polymorphism are shown for at **510 - 530 nm / green** and **550 - 560 nm / yellow** (choose corresponding channel of your real time PCR instrument). The **positive control (red)** contains template **heterozygous** for T/C (-13910) polymorphism (one allel carries the T, another allel carries the C).

Following **figures** shows typical **examples** for **homozygous** as well as **heterozygous** samples on the LightCycler 2.0. Use a appropriate color compensation file, if necessary e.g. LightCycler.

C-Allele at 530 nm



T-Allele at 560 nm



10 Troubleshooting

No fluorescence peak with positive control or samples at about **510-530 nm or 550-560 nm:**

- Proof PCR-program of the real time PCR instrument in use:

⇒ repeat analysis with corrected protocol.

- MutaPLATE® Laktase (TAQ-Man) kit was thawed/ frozen more than twice or stored longer than four days at 2-8 °C:

⇒ consider storage recommendations. Repeat analysis with new MutaPLATE® Laktase (TAQ-Man) reagents.

- low quality of DNA -template:

⇒ exactly follow the manufacturer`s manual for DNA extraction.

Low fluorescence peak at about **510 - 530 nm or 550 - 560 nm**:

- mix single components carfully before use (only by pipetting several times - do not vortex!).
- cool all stock solutions during the working steps in suited manner and protect the detection mix from light.
- Working on ice or with cooled (4°C) Block is recommended.

distributed in the US/Canada by:

Eagle Biosciences, Inc.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 • FAX: 617-419-1110

www.EagleBio.com • info@eaglebio.com

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.