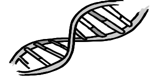
MutaGEL Laktase (AS)



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1. Intended Use

Code: KE09009

The kit "**MutaGEL® Laktase (AS)**" allows the detection of the common -13910 T/C polymorphism in the lactase gene by direct allel-specific detection of genotype. For in vitro diagnostic use only.

2. Introduction

Patients with lactose intolerance are not able to digest milk sugar taken in with the food. Due to this fact this persons suffer subsequently under malabsorption problems like nausea, flatulence, diarrhoea or stomach pain. The most important reason of lactose intolerance is founded in a genetically caused lack of the enzyme lactase which is responsible for the degradation of milk sugar in the organism. This common gene defect is very easy to detect by analysing the T/C base replacement at position -13910 from the regulatory region of lactase gene (LCT). If this base pair exchange from T to C is homozygous, a lactase-deficiency and subsequently lactose intolerance is predetermined. The manifestation of the disease occurs with about 20 years and the prevalence of the homozygous C / C – genotype in Germany is about 15 %, in Mediterranean countries up to 50%.

3. Test Principle

The kit „MutaGEL® Laktase (AS)" is an amplification refractionary mutation system (ARMS) containing two sets of primers for both allelspecific sequences within the lactase gene. The sequence specific primers can be used directly for PCR with extracted DNA. The resulting amplification products are subsequently identified with gelelectrophoretic methods. If there is no specific allel-product detectable, the correct PCR procedure is proved by an internal control. The present genotype of unknown samples is interpreted by detection of corresponding DNA-amplificates for wildtype- and mutation- constellation in two separate lanes of the gel (method by Dr. Essrich, Biologisches Labor, Denzlingen).

4. Materials Supplied (for 24 determinations)

- PCR-**Mix 1** "Primer **C**" 1 x 550 µl **(green)** - ready to use PCR reagent (*hot start* Taq enzyme, $MgCl_2$, dNTP, buffer) with oligonucleotides specific for **C**-allel of the human lactase gene.
- PCR-**Mix 2** "Primer **T**" 1 x 550 µl **(liliac)** - ready to use PCR reagent (*hot start* Taq enzyme, $MgCl_2$, dNTP, buffer) with oligonucleotides specific for **T**-allel of the human lactase gene.
- positive control DNA 1 x 30 µl **(red)** - solution with DNA of heterozygous **C/ T**- variant of -13910 lactase gene

5. Material Required but not Supplied

Reagents:

- DNA extraction kit (e. g. Code.: KBR3005)
- reagents for gel electrophoresis

Instruments:

- thermal cycler (and optional mineral oil or PCR-wax for cycler without heated lid)
- pipettes (0.5 - 200 µl) and sterile pipette tips (with filter)
- sterile micro tubes suitable for the thermal cycler in use
- instruments for gel electrophoresis

6. Storage and Stability

Store at ≤ -18°C. The reagents are stable in the unopened micro tubes until the expiration date indicated (see print on the package).
Before use: Spin tubes briefly before opening (contents may become dispersed during shipment).

7. Warnings and Precautions

- For in vitro diagnostic use only.
- Test should only be performed only by skilled persons considering GLP (Good Laboratory Practice) guidelines.
- Don't use the kit after its expiration date.
- After usage, dispose all reagents and test components included in the kit in conventional garbage.
- PCR technology is extremely sensitive. The amplification of a single DNA molecule generates million identical copies. Therefore set up three separate working areas for a) sample preparation, b) PCR reagent preparation and c) DNA detection. For each working area a different set of pipettes should be reserved.
- Wear separate coats and gloves in each working area.
- Use sterile filter tips for pipetting and use special PCR pipettes for aerosol free pipetting.
- Routinely decontaminate your pipettes and the laboratory benches.
- Avoid aerosols.

8. Procedure

The complete procedure is divided in three steps:

1) Sample preparation.
2) Amplification with two sets of primers allel-specific for the lactase gene.
3) detection of the amplified DNA by gel-electrophoresis and subsequent analysis of genotype.

9. Sample preparation

- Extract total genomic DNA (e. g. from 200 µl whole blood) using a commercial available DNA extraction kit according to the manufacturer`s manual.
- Start immediately with the amplification procedure or store the extracted DNA at ≤ -18°C.

10. Amplification

- Every set of amplifications should include a positive and a negative control.
- Prepare for each sample, positive control and negative control the following Master-Mix for each reaction with the "**C**"-base as well as with the "**T**"-base in parallel (multiply the volumes necessary for each reaction with the number **N** of reactions and add one more volume).

PCR- reagent	reaction- volume: 25 µl	Master-Mix- volume
PCR-Mix	20 µl	20 µl x (N + 10%)

- Aliquot 20 µl of the Master-Mix "**C**-Primer" (**Mix 1**) respectively "**T**-Primer" (**Mix 2**) in two separate (sterile) micro tubes suitable for thermal cyclers.
- Samples: add 5 µl of the **extracted DNA** to each of both Master-Mixes.
- Positive control: add 5 µl of the **positive control DNA** to each of both Master-Mixes (the control DNA is heterozygous (C/T) and therefore suited for both reagents in Mix 1 and Mix 2).
- Negative control: add 5 µl of **primer buffer** to each of both Master-Mixes.
- Transfer the micro tubes into the thermal cycler (if necessary overlay the Mix with 60 µl of mineral oil).
- Perform exactly the following amplification protocol:

Initial hold:	94°C for 5 min
37 cycles:	**94°C** for 30 seconds / **58°C** for 30 seconds / **72°C** for 30 seconds
Final hold:	72°C for 5 minutes, 4°C follow up

11. Analysis of Genotype and Interpretation of Results

- Carry out a gel electrophoresis in **2.5 - 3 %** agarose (or polyacrylamide 20 %) for about **100 Vh** (e. g. 60 min at 100 volts): mix about **15 µl** of each digestion mix with **4 µl** loading buffer (e. g. KAN01070) and load the gel. The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard (e. g. KBR311005). The separated DNA is colored by ethidium bromide or SybrGreen (5 µg/ ml) for 5 min and visualised under UV-light (312 nm).
- The PCR amplification leads for both allels (f. e.: positive control) and all samples to DNA-fragments of **170 bp.**
- Additionally an **internal control** fragment for PCR performance is detectable at **400 bp**. (*Please consider:* if the diagnostic relevant fragment of 170 bp is present, the internal control can be weaker or even absent).
- The presence (**+**) of base "**C**"-base is indicated by detection of the DNA-fragment (170 bp) in **Mix 1** ("C"-Primer), whereas the presence of "**T**"-base is indicated by detection of the DNA-fragment (170 bp) in **Mix 2** ("T"-Primer). Therefore the following pattern are possible:

GENOTYPE	PCR-Mix 1 (C-Primer)	PCR-Mix 2 (T-Primer)
C / C	**+**	**--**
C / T	**+**	**+**
T / T	**--**	**+**

- The **positive control DNA** possesses for the analysed -13910-polymorphism of LCT-gene the genotype **C / T** (= **heterozygous**).
- The **internal amplification control** appears at **400 bp**.
- In any case the negative controls must be negative for any amplification product of indicated lengths.

12. Restrictions

The PCR results for positive control in DNA fragments of indicated length (170 bp) and for samples at least one amplification product (170 bp) must appear in one of both PCR-Mixes. If this is not the case, the sample must be tested a second time or the complete analysis must be repeated with freshly isolated DNA. If there are neither positive control DNA fragments nor the internal control fragment (400 bp) present, the amplification was incorrect and the chosen PCR conditions have to been proven/ corrected.