MutaGEL Aldolase B

(Fructosemia A150P – allel specific)

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1. Intended Use	Code: KE09013

The kit "**MutaGEL® Aldolase B** (fructosemia A150P - allelspecific)" allows the detection of the comon mutation "codon 150P" in the human fruct-aldolase (aldolase B) gene by direct allelspecific detection of genotype.

2. Introduction

The liver isoenzyme Aldolase B is critical for sugar metabolism, and a catalytic deficiency due to mutations in its gene may result in hereditary fructose intolerance (HFI) syndrome, with hypoglycaemia and severe abdominal symptoms.

The autosomal recessive disorder HFI is a potentially lethal inborn error in metabolism and the disease poses diagnostic problems because of in part very unspecific clinical manifestations. The present aldolase B PCR test is useful for the detection of the three most common mutations critical for gluconeogenesis and fructose metabolism: A149P (60%), A174D (11%) and N334K (8%). These mutations may account together for more than 80% of all known mutations causing HFI and their screening will be helpful for suited therapy of afflicted patients.

The G>C mutation at bp position 448 results in an amino-acid exchange (from alanine to praline) at position 150 (earlier 149). Frequency of that mutation is 1:250 in Caucasians and disease by homozygous presence of C-allel is about 1:50000 (=0.002%).

3. Test Principle

The kit „MutaGEL® Aldolase B (AS)" is an amplification refractionary mutation system (ARMS) containing two sets of primers for both allelspecific sequences within the fructaldolase 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 normal (G) - and mutation (C) - 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 **G**)	1 x	550 µl	**(green)**	- ready to use PCR reagent (*hot start* Taq enzyme, $MgCl_2$, dNTP, buffer) with oligonucleotides specific for **normal sequence (A)** in codon 150 (= **G**448).
▪ PCR-**Mix 2** (Primer **C**)	1 x	550 µl	**(liliac)**	- ready to use PCR reagent (*hot start* Taq enzyme, $MgCl_2$, dNTP, buffer) with oligonucleotides specific for **mutation sequence (P)** im codon 150 (= 448**C**).
▪ Positive control DNA	1 x	50 µl	**(red)**	- DNA solution heterozygous for codon A150P of aldolaseB gene (= **G**448**C**).
▪ Negative control	1 x	100 µl	(transparent)	- buffered solution for DNA free control

5. Material Required but not Supplied

Reagents:

- DNA extraction kit (e. g. MutaCLEAN® DNA Blood, Code: KG1033)
- reagents for gel electrophoresis

Instruments:

- thermal cycler
- 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). Don't thaw out the content of the "positive control DNA" for more than two times. If necessary, make suitable aliquots.

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.

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8. Procedure

The complete procedure is divided in three steps:

1) Sample preparation (DNA extraction).
2) Amplification with two sets of primers allel-specific for the aldolase B - 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 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 "**G**"-base as well as with the "**C**"-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 "**G**-Primer" (**Mix 1**) respectively "**C**-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 (G/C) 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 15 min
37 cycles:	**95°C** for 30 seconds / **56°C** for 30 seconds / **74°C** for 30 seconds
Final hold:	72°C for 10 minutes, 4°C follow up

11. Analysis of Genotype and Interpretation of Results

- Carry out a gel electrophoresis in **2,5 %** agarose (or polyacrylamide 20 %) for about **120 Vh** (e. g. 80 min at 90 volts): mix about **15 µl** of each digestion mix with **4 µl** loading buffer and load the gel. The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard. 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 the postitve control to DNA-fragments of **130 bp** (normal) with **Mix 1** and of **200 bp** (mutated) with **Mix 2**.
- Additionally an **internal control** fragment for PCR performance is detectable at **400 bp**.
- The presence (**+**) of base "**G**" in the patient samples is indicated by detection of the corresponding DNA-fragment (**130 bp**, normal) in **Mix 1** ("**G**"-Primer), whereas the presence of "**C**"-base in the patient samples is indicated by detection of the DNA-fragment (**200 bp**, mutated) in **Mix 2** ("**C**"-Primer). Therefore the following pattern are possible:

GENOTYPE	PCR Mix 1 (A150)	PCR Mix 2 (150P)
G / C	**+**	**--**
G / C	**+**	**+**
C / C	**--**	**+**

- The **positive control DNA** possesses for the analysed G448C polymorphism of aldolase B - gene the genotype **G / C** (= 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 and for samples at least one amplification product 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.

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