MutaGEL® α1 Antitrypsin (S/Z)

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1. Intended Use

Code: KE09014

The kit "MutaGEL®α1Antitrypsin S+Z"- allows the analysis of the frequent mutations „S" and "Z" in the human α1antitrypsin gene. For in vitro diagnostic use only.

2. Introduction

The antiproteinase α1antitrypsin protects in general own organs (as lung and liver) from „self-digest" by antagonistic regulation of protein-degrading enzymes from bacteria-defense and metabolism. Persons with hereditary decreased antitrypsin activity (this protein contains the highest antiproteinase concentration in the human organism) are often afflicted with in part heavy (liver) diseases. This deficiency (measurable in the human serum) is caused by several different base exchanges in the α1antitrypsin gene.Most iImportant is the homozygous constellation of "Z"-mutation (amino-acid Glu to Lys in codon 342 of exon 5) and the more rare "Zero-mutations" which are dispersed over different regions in the gene leading to no enzyme activity.

In contrary, the "S"-mutation (Glu to Val in codon 246 of exon 3) has protective properties: S-homozygotie and also compound heterozygotie with the Z-mutation (SZ) does not cause liver diseases. The enzyme product of the "S"-mutation has a short half-life and consequently a decreased serum activity. Hereditary antitrypsin deficiency with organ-defects is characterized by lung emphysema or chronic hepatitits (resp. liver cirrhosis or hepato-cellular carcinoma) and is in Europe with a frequence of 1: 2-5000 the main cause for the mentioned hereditary liver diseases by children.

3. Principle of the test

This PCR test allows the diagnosis of the „S" and "Z" mutations using two different PCR techniques unified in the kit MutaGEL®α1Antitrypsin S+Z: First, allel-specific primer pairs are used for identification of "S"-mutation site second, an RFLP (restriction length polymorphism) PCR-method is used for identification of the "Z" mutation. Result-interpretation is done by subsequent detection of generated DNA products/ fragments by gel-electrophoresis.

4. Material Supplied (for 24 determinations)

▪ PCR Mix (S Normal)	1 x 550 µl (**green**)	solution of ready to use Master Mix with oligonucleotides specific for "normal S" sequence
▪ PCR Mix (S Mutation)	1 x 550 µl (**violet**)	solution of ready to use Master Mix with oligonucleotides specific for "mutatedl S" sequence
▪ PCR Mix (Z)	1 x 555 µl (**yellow**)	solution of ready to use Master Mix with oligonucleotides specific for complete "Z" region
▪ Restriction enzyme (Z)	1 x 20 µl (**blue**)	restriction enzyme for the polymorphism in the „Z" region
▪ Restriction buffer (Z)	1 x 280 µl (transp.)	buffer for restriction enzyme (Z)
▪ Positive control DNA	1 x 30 µl (**red**)	positive control DNA for „S" resp. „Z" - mutation of α1antitrypsin gene

5. Materials Required but not Supplied

- DNA extraction kit (e.g. Code: KBR3005)
- PCR water (pure)
- thermal cycler + mineral oil or wax (optional for thermal 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
- reagents and 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). Avoid longer storage at 4 - 8°C in the refrigerator: freeze back again after usage for long-time storage.
Before use: Spin tubes briefly before opening to collect all solutions at the bottom of the tube (contents may become dispersed during shipment).

7. Warning 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 and special PCR pipettes for aerosol free pipetting.
- Routinely decontaminate your pipettes and the laboratory benches.
- Avoid aerosols (especially during opening of the reagent tubes).

Procedure

The complete procedure is divided in four steps:

1. Sample preparation (extraction of genomic DNA).
2. Amplification with both primer-sets specific for the α1antitrypsin gene (each for one of the both polymorphisms).
3. Digestion of the amplified "Z" product with the restriction enzyme mix.
4. Detection of the amplified ("S") and digested ("Z") DNA by gel electrophoresis (size resolution).

8. Sample Preparation

For template use total genomic DNA which can be extracted (e. g. from 200 µl of whole blood) using commercial available DNA extraction kits according to the manufacturer`s manual. Start immediately with the amplification procedure or store the extracted DNA at ≤ -18°C.

9. Amplification

- For each sample are prepared two parallel reactions for the „S"-mutations and (only) one reaction for the amplification of „Z"-region.
- Every set of amplifications should include a positive control (PC in the kit is "SZ" and therefore suited for both polymorphisms) as well as a negative control.
- For each single sample, positive/ negative control aliquot **20 µl** of each PCR ready to use Mix in sterile PCR-tubes using following scheme for Master Mix preparation:

PCR- Reagents	Reaktion- Volume: 25 µl	Master Mix- Volume 20µl
S-mutation: for each of the both base detection prepare one specific reaction (in summary 2):	per single reaction	per „N" samples
PCR Mix "**S normal**"	**20 µl** + 10%	**N** x **20 µl** + 10%
PCR Mix "**S mutation**"	**20 µl** + 10%	**N** x **20 µl** + 10%
Z-mutation: for each sample prepare only 1 reaction:		
"PCR Mix Z"	**20 µl** + 10%	**N** x **20 µl** + 10%

- **Samples**: add **5 µl** of **extracted DNA** as template to each Master-Mix.
- **Positive Control**: add **5 µl** of the antitrypsin **positive control DNA** to the corresponding Master Mix (the positive control included in the kit is made with "SZ"-constellation and therefore useful for all control reactions).
- **Negative control**: add 5 µl of **primer buffer** or H_2O to the corresponding MasterMix.
- Transfer the prepared PCR-tubes to the thermocycler (if necessary, add about 60 µl mineral oil or wax).
- Perform the following PCR amplification protocol:

Initial phase:	**95°C** for 5 min
37 Cycles:	**95°C** for 30 sec / **58°C** for 30 sec / **72°C** for 30 sec
Final phase:	**72°C** for 5 min, **10°C** follow up

10. Restriction enzyme digest for detection of „ Z"-allel (reaction volume: 20µl)

Per sample: prepare **10 µl** restriction Mix:	per single reaction	per „N" samples
	9 µl restriction buffer Z + 10% + 0,7 µl restriction enzyme Z	N x 9 µl restriction buffer Z + 10% + N x 0,7 µl restriction enzyme Z
Add to each **10 µl** prepared reaction Mix 10 µl of amplified Z-DNA and incubate for **>3h** or optimal over night at **65°C**.		

11. Detection of the amplified DNA and Interpretation of the Results

- Carry out a gel electrophoresis in **2 %** agarose (or 10% polyacrylamide) for about **100 Vh** (e.g. 70 min 116 volts) in 1x TBE-Buffer: mix about **15 µl** of each PCR-reaction with **4 µl** loading buffer (for each extracted DNA are two lanes necessary in order to detect the DNA fragments generated from both primer mixes (**A** and **B**) = two lanes per patient). The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard (e. g. KBR311005). The separated DNA is colored in an **ethidium bromide**- or **SybrGreen**- (5 µg/ ml) bath for about **5 - 10 min** and visualised under **UV-light (312 nm)**. The received DNA-band pattern could be (foto-) documented.
- The "S"-PCR generates for "**S-normal**" allel a DNA fragment of **200 bp** length and for the "**S-muation**" allel an **150 bp** – fragment.
- The "Z"-PCR generates an amplification product of 135 bp. The normal gene is cutted by restriction enzyme in 90 + 45 bp fragments. The alleles with "Z-mutation" have no restriction site for the enzyme and stay un-cutted.

Both PCRs contain an **internal control** (human ß-globin gene) leading to a DNA fragment of **400 bp**.

GENOTYPE	PCR Mix "S-normal"	PCR Mix "S-mutation"
	200 bp + 400 bp control band	150 bp + 400 bp control band
GENOTYPE	**PCR Mix „Z" after restriction: Normal**	**PCR Mix „Z" after restriction: Z-Mutation**
	135 bp + 400 bp control band	90 bp (+ 45 bp) + 400 bp control band

- The **antitrypsin positive control DNA** is genotype „**SZ**".
- amplification of negative control does not result in DNA bands of indicated fragment lengths.

12. Restrictions

The PCR results for all positive controls in DNA fragments of indicated length and for samples at least in the internal control fragment (400 bp). 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 no positive control DNA fragments present, the amplification was incorrect and the chosen PCR conditions have to been proven/ corrected.

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