MutaGEL® GST-P1

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1. Intended Use

Code: KV0903100

The kit MutaGEL® GST-P1 allows the detection of the Ile105Val polymorphism in the human glutathione-S-transferase gene (GST-P1). For in vitro diagnostic use.

2. Introduction

The glutathione-S-transferase system protects against peroxides and electrophilic reaction partners which both can damage the cells of the body. The enzyme exists in several different isoforms. The solubility of the (manifold) substrates is increased by the addition of glutathione leading to a better elimination of the toxic metabolites from the body. Glutathione-S-transferase deficiency can be genetically determined. The GST-P1 gene compensates (at least in parts) for the frequently deletion of GST-M1 or GST-T1 genes in Caucasians (about 50 % respectively 20 %).

3. Test Principle

The kit "MutaGEL GST-P1" contains a set of primer which amplify a specific sequence within the human GST-P1 gene (primer GST-P1). The amplified product obtained from a wild type DNA will not be cut by the restriction enzyme included in this kit, whereas the fragment obtained from DNA carrying the Il105Val-mutation will be cut once. The identification of the present genotype is done by analysis of the amplification products and their cut fragments through gel electrophoresis.

4. Materials Supplied (for 24 determinations)

▪ PCR Master Mix	1 x 540 µl (**grün**)	ready to use PCR Mix (buffer solution, *hotstart*-Taq, dNTP, Mg^{2+}, oligonukleotides specific for the human GST-P1 gene.
▪ Positive control DNA	1 x 35 µl (**rot**)	buffered solution with (amplified) heterozygous DNA of human GST-P1 plus control DNA.
▪ Restriction enzyme G	1 x 70 µl (**blue**)	restriction enzyme for GST-P1 mutation site.
▪ buffer for restriction	1 x 540 µl (white)	buffer for the restriction enzyme G.

5. Material Required but not Supplied

Reagents:
- DNA extraction kit (f.e. Code.: KDBR3005)
- reagents for gel electrophoresis (agarosis, running buffer TB, molecular weigt standard, loading buffer, ethidium bromide)
- mineral oil (optional, for thermal cyclers without heated lid)

Instruments:
- thermal cycler
- pipettes (0.5 - 200 µl) and sterile pipette tips (with filter)
- sterile reagent tubes and PCR micro tubes suitable for the thermal cycler in use
- instruments for gel electrophoresis (power supply, running chamber)

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 "GST-P1 positive control DNA" for more than three times. If necessary, make suiteable aliquots.
Before use: Spin tubes briefly before opening (contents may become dispersed during shipment).

7. Warnings and Precautions

- For in vitro diagnostic use.
- 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.
- Routinely decontaminate your pipettes and the laboratory benches.
- Avoid aerosols (use sterile filter tips for pipetting and use special PCR pipettes with filter for aerosol free pipetting)

Procedure

The complete procedure is divided in four steps:
1) Sample preparation.
2) Amplification with primers specific for the human GST-P1 gene.
3) Digestion of the amplified product with a restriction enzyme.
4) Detection of the amplified and digested DNA. by gel-electrophoresis.

8. Sample preparation

- Extract total genomic DNA e.g. from 200 µl of 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.

9. 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 (multiply the volumes necessary for each reaction with the number **N** of reactions and add 10% more volume).

PCR Reagents	Reaction volume: 25 µl	Master-Mix volume
PCR Mix volume (inclusive primers)	20 µl	20 µl x (N + 10%)

- Per each analysis: aliquot **20 µl** of PCR Master-Mix in sterile micro tube suitable for the thermal cycler
- Samples: add **5 µl** of the **extracted DNA** to the PCR Master-Mix
- Positive control: add **5 µl** of the **GST-P1 positive control DNA** to the PCR Master-Mix
- Negative control: add **5 µl** of **H_2O** (PCR grade) to the PCR Master-Mix
- Transfer the micro tubes into the thermal cycler (if necessary overlay the Mix with 60 µl of mineral oil)
- Perform the following amplification protocol:

Initial hold:	95°C for 5 min
37 cycles:	95°C for 30 seconds / 58°C for 30 seconds / 72°C for 60 seconds
Final hold:	74°C for 10 minutes, 4°C follow up

10. Digestion of the Amplified DNA

Prepare for each sample, including positive control the following Digestion-Mix (multiply the volumes necessary for each reaction with the number **N** of reactions, and add 10% more volume).

Reagents for DIGESTION	Total volume for each DIGESTION: 30 µl	Volume in the Digestion-Mix
Buffer Mix for restriction enzyme G	19.6 µl	19.6 µl x (N + 10%)
Restriction enzyme G	2.4 µl	2.4 µl x (N+1)

- aliquot **15 µl** of the Digestion-Mix into reaction tubes suitable for heating block incubator (a thermal cycler may be used for the incubation too).
- add **15 µl** of the amplification product to the digestion Mix.
- transfer the reaction tubes to the heating block incubator.
- incubate at **55°C** for minimal **3 hours** (optimal over night).

11. Detection of the Amplified and Digested DNA

- Carry out a gel electrophoresis in **2 - 3 %** agarose (or polyacrylamide 20 %) for about **65 Vh** (e.g. 40 min 90 volts): load about **15 - 20 µl** of each restriction (with about **4 µl** loading buffer) to the gel in order to obtain a complete separation of the different fragments. The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard (e.g. KDGC-015001). The separated DNA is colored by ethidium bromide (5 µg/ml) for about 5 min and visualised under UV-light (312 nm).
- The amplification leads (without restriction) to a GST-P1 specific fragment of **180 bp** length. Additionally a **400 bp** control amplimer is generated.
- The mutation in the GST-P1 gene leads to a modified amplification product with a sequence recognised be the restriction enzyme G. Subsequently it will be cutted in case of present mutation and stay complete in case of normal sequence. Furthermore, the control amplimer contains a restriction site which shortens the amplimer by 40 bp (to 360 bp) after restriction. Therefore, the following restriction enzyme patterns are obtained:

GENOTYPE	Length of the digested DNA (in base pairs)
wt / wt	**180** / **-** / **-** (+ 360 bp control fragment)
wt / **mut**	**180** / **100** / **80** (+ 360 bp control fragment)
mut / **mut**	**-** / **100** / **80** (+ 360 bp control fragment)

- The **GST-P1 positive control DNA** has the genotype **wt/mut.**
- In any case the negative controls must be negative for any amplification product.

12. Restrictions

The PCR results for all positive controls in DNA fragments of indicated length and for samples at least in the amplification product of 180 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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