



1. Intended Use

Code.: KV0902100

The kit "MutaGEL GST-M1/ T1" allows the simultaneous detection of the glutathione-S-transferase-genes GST-M1 and GST-T1 (and additionally the albumin gene as internal control).

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: more than 50% of Caucasians do not carry the GST-M1 gene and about 20% do not have the GST-T1 gene.

3. Test Principle

The kit "MutaGEL GST-M1/ T1" contains a set of primer which amplify a specific sequence within the human GST-M1 and respectively the GST-T1 gene as well as within the albumin gene (primer GST-M1/ T1/ albumin):

- GST-M1: if the gene is present a fragment of 216 bp will be amplified.
- GST-T1: if the gene is present a fragment of 474 bp will be amplified.
- albumin: the amplification of the 350 bp fragment of the albumin gene is used as internal control for each PCR reaction.

4. Materials Supplied (for 24 determinations)

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|------------------------------|------------|---|
| ▪ primer GST-M1/ T1/ albumin | 1 x 80 µl | solution of oligonucleotides specific for the human genes GST-M1, GST-T1 and albumin. |
| ▪ primer buffer | 1 x 2.0 ml | buffered aqueous solution, used also as negative control. |
| ▪ dNTP-Mix | 1 x 30 µl | solution of the four dNTPs. |
| ▪ positive control DNA | 1 x 30 µl | aqueous solution of human DNA with the (cloned) DNA of GST-M1, GST-T1 and albumin. |

5. Material Required but not Supplied

Reagents:

- DNA extraction kit (f.e. Code.: KDBR3005)
- reagents for gel electrophoresis
- Taq polymerase (5 U / µl ; f.e. Code : KDTO100)
- Taq reaction buffer (10 x, with 15 mM MgCl₂)
- mineral oil (for thermal cyclers without heated lid)

Instruments:

- thermal cycler
- pipettes (0.5 - 1000 µl) and sterile pipette tips
- 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 "GST-M1/ T1/ albumin 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.
- PC R 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.



Procedure

The complete procedure is divided in three steps:

- 1) Sample preparation.
- 2) Amplification with primers specific for the GST-M1/ T1 genes.
- 3) Detection of the amplified DNA.

8. Sample Preparation

- Extract total genomic DNA f.e. from 200 µl whole blood using a commercial available DNA extraction kit according to the manufacturers 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 one more volume).

PCR Reagents	reaction volume: 45 µl	Master-Mix volume
primer buffer	35.5 µl	35.5 µl x (N+1)
Taq- reaction buffer (10 X)	5 µl	5 µl x (N +1)
dNTP Mix	1 µl	1 µl x (N+1)
GST-M1/ T1/ abumin - primer	3 µl	3 µl x (N+1)
Taq polymerase	0.5 µl	0.5 µl x (N+1)

- aliquot 45 µl of the Master-Mix in sterile micro tube suitable for the thermal cycler.
- samples: add 5 µl of the **extracted DNA** to the Master-Mix
- positive control: add 5 µl of the **GST-M1/T1/albumin positive control DNA** to the Master-Mix
- negative control: add 5 µl of **primer buffer** to the Master-Mix
- if necessary overlay the Mix with 60 µl of mineral oil
- transfer the micro tubes into the thermal cycler
- perform the following amplification protocol:

Initial hold:	94°C for 4 min
35 cycles:	94°C for 60 seconds / 58°C for 45 seconds / 72°C for 45 seconds
Final hold:	72°C for 5 minutes, 4°C follow up

10. Detection of the Amplified DNA and Interpretation of the Results

- Carry out a gel electrophoresis in 2 % agarose (or 20 % polyacrylamide gel) with about 10-15 µl of the amplified material in order to obtain a complete separation of the DNA fragments. The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard. The separated DNA is colored by ethidium bromide (5 µg/ml) for 5 min and visualised under UV-light (312 nm).
- The use of 5x TBE running buffer (Code: KAN10060), 6x loading buffer (Code: KAN01070), molecular weight marker pUC19/ *MspI* (KBR311005) and (in case of using pre-cast gels) polyacrylamide gels (Code: KAN20112) is recommended.
- The amplification produces the following fragments:

GENOTYPE	Length of the amplified DNA (in base pairs)		
M1+/T1+	216	474	(+ 350)
M1+/T1-	216	-	(+ 350)
M1-/T1+	-	474	(+ 350)
M1-/T1-	-	-	(+ 350)

- The "GST-M1/ T1 positive control DNA" has the genotype **M1+ / T1+** (both alleles are present).
- In any case the negative controls must be negative for any amplification product.
- The albumin amplification control generates a 350 bp- fragment which must be present in each reaction (except the negative control reaction).

11. Restrictions

The PCR results for all positive controls in DNA fragments of indicated length and for samples at least in the internal control fragment (350 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 be proven/ corrected.

