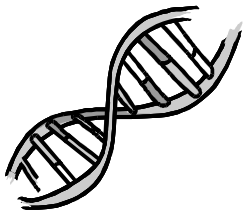

MutaPLATE® GSTP1 (TM)

PCR test for analysis of the Ile105Val polymorphism of the GSTP1 on open Real-Time PCR systems (e.g. RotorGene, SmartCycler, Light Cycler, ABI, MyGo Pro, Stratagene) by TaqMan technology

IVD

for in-vitro diagnostic use only

CE

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1 Intended Use

The MutaPLATE GSTP1 (TM) Real-Time PCR kit allows the detection of the Ile105Val polymorphism in the GSTP1 gene from genomic DNA. The test is based on the TaqMan technology and can be used with all open Real-Time PCR instruments (e.g. RotorGene, SmartCycler, LightCycler, ABI, Stratagene, MyGo Pro).

2 Introduction

The glutathione-S-transferase (GST) are classified into a multitude of classes and play a key role in the cellular detoxification, as they regulate the conjugation of toxic substances into their excretable metabolites. Therefor the individual enzyme activity influences the sensitivity to environmental toxins, carcinogens, cancer and other diseases.

3 Concept of the Assay

The assay contains two sequence specific primers flanking the region of interest and two TaqMan probes specific to the region containing the mutation. The two TaqMan probes are labeled at the 5' end with different fluorophores (reporter dyes) which are used for the allelic discrimination. On the 3' end the TaqMan probes are labeled with a non-fluorescent quencher. The proximity of the reporter dye to the quencher inhibits the fluorescence of the reporter molecule. During amplification the probes hybridize specifically to the DNA fragments. The 5' nuclease activity of the Taq polymerase cleaves the hybridized probes releasing the reporter from the quencher generating a fluorescent signal.

4 Kit Components

GSTP1 Ile105Val Kit	**Volume**	
Reagent	**32-rxn**	**96-rxn**
Enzyme Mix **(blue lid)**	438 µL	2 x 660 µL
Detection Mix GSTP wt **(yellow lid)**	175 µL	3 x 175 µL
Detection Mix GSTP mut **(white lid)**	175 µL	3 x 175 µL
Positive Control **(red lid)**	15 µL	45 µL
Negative Control **(green lid)**	150 µL	150 µL

5 Required Materials

Required Materials - not provided:

- Open Real-Time PCR system
- 200 µL PCR tubes (sterile)
- Cryo container for PCR reaction tubes
- Pipettes (0.5 – 200 µl)
  - 0.5 - 10 µL
  - 10 - 200 µL
- 1.5 mL reaction tubes
- PCR H2O, sterile, DNase-free, DNA-free, Molecular biology grade, RNase-free

6 Storage and Handling

- All reagents should be stored at -20 °C until immediate use.
- Avoid several freeze / thaw cycles for the reagents (if necessary prepare aliquots)
- The detection mixes have to be protected against exposure to light

7 Consideration and Precautions

The regulations and principles for working in a biomolecular laboratory have to be strictly followed.

- All steps have to be performed in an uninterrupted manner
- All PCR reagents have to be cooled while working
- The DNA purity (A260/A280 ratio) should be between 1.8 and 2.0

8 Test Procedure

8.1 PCR Preparation

Gently thaw all components on ice and gently mix them before use (do not vortex) and shortly spun down. Keep in mind to protect the detection mixes against exposure to light. During the PCR setup all reagents have to be cooled.

For the amplification one PCR tube (or a reaction tube corresponding to the used Real-Time instrument) is needed per sample plus two additional tubes for the negative and positive controls. The following table shows the volume of each reagent per sample. For the analysis a mastermix should be prepared for the number of samples (incl. negative and positive control) (N) plus 10 % to compensate inaccuracies. The mastermix should be prepared in the same order as indicated in the table on the next page.

Reagent	Volume	Master Mix Volume
Detection Mix GSTP wt (yellow lid)	5 µL	5 µL * (N + 0.1)
Detection Mix GSTP mut **(white lid)**	5 µL	5 µL * (N + 0.1)
PCR H2O*	0.5 µL	0.5 µL * (N + 0.1)
Enzyme Mix **(blue lid)**	12.5 µL	12.5 µL * (N + 0.1)

*not provided w ith kit

- The mastermix has to be carefully mixed through by pipetting up and down or inverting (do not vortex) and shortly spin down. Aliquot 23 µL into each PCR tube.
- For the negative control add 2 µL of the provided negative control **(green lid)**.
- For the positive control add 2 µL of the provided positive control DNA **(red lid)**.
- Add 2 µL of each sample DNA to the corresponding PCR tube.

The PCR reactions have to be carefully mixed through and shortly spun down. Subsequently place them into the Real-Time PCR instrument and use the PCR protocol described in 8.2.

8.2 PCR Protocol

Step	Temperature [°C]	Time [s]	Cycles	Acquisition
Lid Heat (if applicable)	99	---	---	---
Initial Denaturation	94	120	1 x	none
Denaturation	94	10	40 x	none
Primer Annealing and Elongation	60	60		**single**
Lid Heat (if applicable)	off	---	---	---
Storage	8	∞	1 x	---

9 Evaluation

The TaqMan probe for the A-allele (wildtype) is marked with FAM **(510 nm, green)** and the TaqMan probe for the G-allele (mutation) is marked with YAK **(555 nm, yellow)**.
Corresponding to the three genotypes following results can be achieved:

1. Homozygous wildtype (A/A):
   Increase of the fluorescent signal from the **FAM** labeled TaqMan probe, no increase of the fluorescent signal from the YAK labeled TaqMan probe.

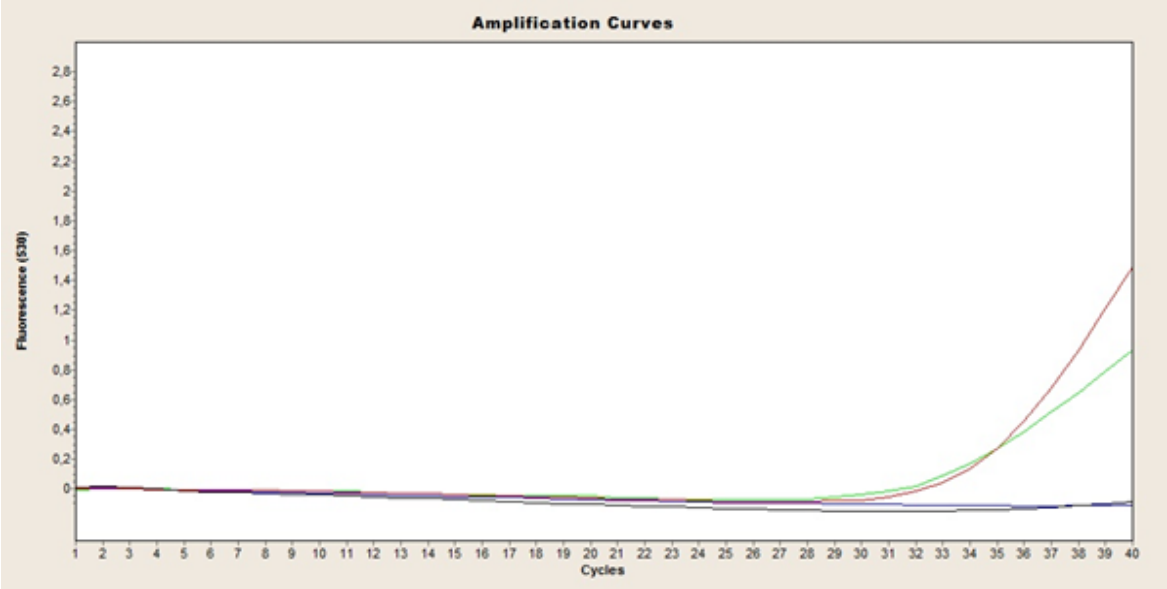
2. Heterozygous mutated (A/G):
   Increase of the fluorescent signal from the **FAM** labeled TaqMan probe and increase of the fluorescent signal from the **YAK** labeled TaqMan probe.

3. Homozygous mutation (G/G):
   No increase of the fluorescent signal from the FAM labeled TaqMan probe, increase of the fluorescent signal from the **YAK** labeled TaqMan probe.

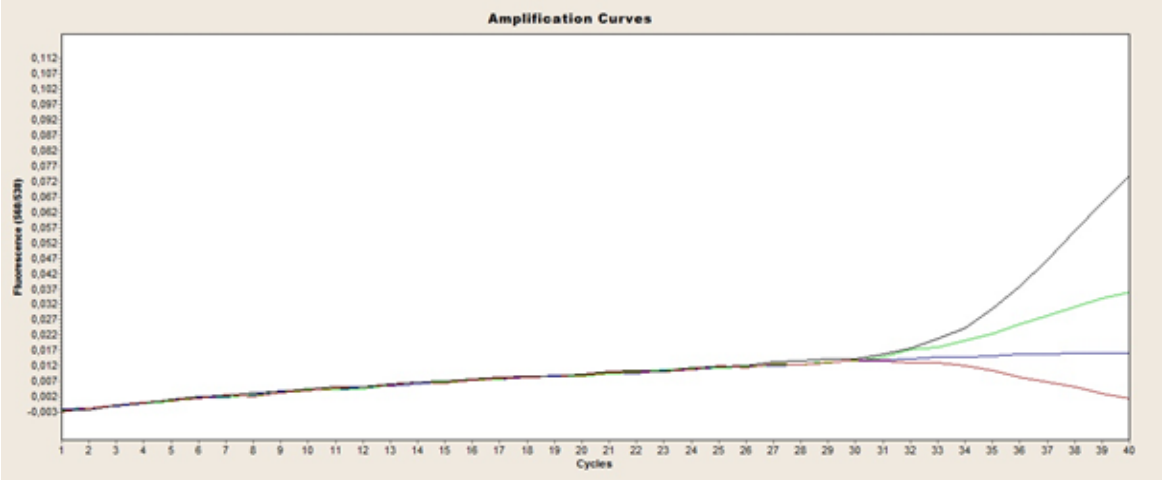
Results of the analysis for the GSTP1 polymorphism are shown for the three possible genotypes at **510 - 530 nm / green** and **550 - 560 nm / yellow** (choose corresponding channel of your Real-Time PCR instrument). Use a appropriate color compensation file, if necessary e.g. LightCycler®.

Following figures show typical results of the experiment performed on the LightCycler® 2.0: **blue curve** - negative control, **red curve** - homozygous wildtype, **green curve** - heterozygous mutation, **black curve** - homozygous mutation.

FAM (530 nm, green) - A allele (wildtype)



YAK (560nm, yellow) - G allele (mutation)



The positive control contains DNA heterozygous for the GSTP1 Ile105Val polymorphism (one allele carries the A, the other carries the G).

10 Troubleshooting

Problem	Solution
No or low fluorescence peak with positive control or samples	Check PCR-program of the real time PCR instrument in use and repeat with corrected protocol
	PCR reagents was thawn / frozen more than twice or stored longer than four days at 2-8 °C. Repeat analysis with a fresh aliquot or new PCR reagents
	Quality of DNA template not sufficient. Use freshly extracted DNA and measure the concentration/purity before use.
	The detection mixes were not protected from light. Repeat analysis with a fresh aliquot or new PCR reagents.