



<b>1. Intended Use</b>	<b>Code: KV0904100</b>
The kit "MutaGEL CYP1A1" allows the detection of the T6235C polymorphism in the CYP1A1-gene encoding for the corresponding cytochrome P450 detoxifying enzyme (phase I).	

<b>2. Introduction</b>
Foreign chemical substances in the human organism are eliminated by own detoxifying enzymes of the body. The inducible cytochrome P450 1A1 (phase I) enzyme is mainly responsible for the activation of polycyclic aromatic hydrocarbons (resulting from burning processes like cigarette smoke or fried meat etc.). The T6235C-mutation in the CYP1A1 gives rise to a higher enzyme activity causing accumulation of toxic metabolites in the body. These are responsible for cell damage and promote the development of cancer. The prevalence of the T6235C mutation in Caucasians is about 4 %.

<b>3. Test Principle</b>
The kit "MutaGEL CYP1A1" contains a set of primer which amplify a specific sequence within the human CYP1A1 gene (primer CYP1). 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 mutation will be cut once. Identification of genotype is done by analysis of amplification products and the cut fragments using gel electrophoresis.

<b>4. Materials Supplied (for 24 determinations)</b>
<ul style="list-style-type: none"> <li>▪ primer CYP1                            1 x 30 µl                            solution of oligonucleotides specific for the human gene CYP1A1.</li> <li>▪ primer buffer                            1 x 2.0 ml                            buffered aqueous solution, used also as negative control.</li> <li>▪ dNTP-Mix                                1 x 30 µl                            solution of the four dNTPs.</li> <li>▪ positive control DNA                1 x 30 µl                            aqueous solution of human DNA with the (cloned) DNA of CYP1A1.</li> <li>▪ enzyme C1                                1 x 60 µl                            restriction enzyme.</li> <li>▪ buffer enzyme C1                       1 x 120 µl                           buffer for the restriction enzyme.</li> <li>▪ BSA solution                             1 x 120 µl                           supplement for restriction.</li> </ul>

<b>5. Material Required but not Supplied</b>
Reagents and instruments: <ul style="list-style-type: none"> <li>▪ DNA extraction kit (f.e. Code.: KBR3005)</li> <li>▪ reagents for gel electrophoresis</li> <li>▪ Taq polymerase with (5 U / µl ; f.e. Code : KDTO100)</li> <li>▪ Taq reaction buffer (10 x, with 15 mM MgCl<sub>2</sub>)</li> <li>▪ mineral oil (for thermal cyclers without heated lid)</li> <li>▪ thermal cycler</li> <li>▪ pipettes (0.5 - 1000 µl) and sterile pipette tips</li> <li>▪ sterile micro tubes suitable for the thermal cycler in use</li> <li>▪ instruments for gel electrophoresis</li> </ul>

<b>6. Storage and Stability</b>
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 "CYP1A1 positive control DNA" for more than two times. If necessary, make suitable aliquots. <i>Before use:</i> Spin tubes briefly before opening (contents may become dispersed during shipment).

<b>7. Warnings and Precautions</b>
<ul style="list-style-type: none"> <li>▪ For in vitro diagnostic use only.</li> <li>▪ Test should only be performed only by skilled persons considering GLP (Good Laboratory Practice) guidelines.</li> <li>▪ Don't use the kit after its expiration date.</li> <li>▪ After usage, dispose all reagents and test components included in the kit in conventional garbage.</li> <li>▪ 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.</li> <li>▪ Wear separate coats and gloves in each working area.</li> <li>▪ Use sterile filter tips for pipetting and use special PCR pipettes for aerosol free pipetting.</li> <li>▪ Routinely decontaminate your pipettes and the laboratory benches.</li> <li>▪ Avoid aerosols.</li> </ul>

<b>Procedure</b>
The complete procedure is divided in four steps: <ol style="list-style-type: none"> <li>1) Sample preparation.</li> <li>2) Amplification with primers specific for the CYP1A1 gene.</li> <li>3) Digestion of the amplified product with a restriction enzyme.</li> <li>4) Detection of the amplified and digested DNA.</li> </ol>



## 8. Sample Preparation

- Extract total genomic DNA f.e. from 200 µl of 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	37.5 µl	37.5 µl x (N+1)
Taq- reaction buffer (10 X)	5 µl	5 µl x (N +1)
dNTP Mix	1µl	1 µl x (N+1)
primer CYP1	1 µl	1 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 **CYP1A1 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:

<b>Initial hold:</b>	94°C for 3 min
<b>35 cycles:</b>	94°C for 30 seconds / 58°C for 30 seconds / 72°C for 30 seconds
<b>Final hold:</b>	72°C for 5 minutes, 4°C follow up

## 10. Digestion of the amplified DNA

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

Reagents for DIGESTION	Total volume for each DIGESTION: 40 µl	Volume in the Digestion-Mix
buffer enzyme C1	4 µl	4 µl x (N+1)
enzyme C1	2 µl	2 µl x (N+1)
BSA solution	4 µl	4 µl x (N+1)

- Aliquot 10 µl of the Digestion-Mix into tubes suitable for the incubator (a thermal cycler may be used for the incubation too).
- Add 30 µl of the amplification product to the digestion Mix.
- Transfer the tubes to the incubator and incubate at **37°C for 3 hours** (optimal over night).

## 11. Detection of the amplified and digested DNA

- Carry out a gel electrophoresis in 2 % agarose (or polyacrylamide 20 %) with about 10-15 µl of the amplified and digested DNA in order to obtain a complete separation of the different fragments.
- 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 leads to a fragment of **340 bp** length.
- The mutation in the CYP1A1 gene leads to a modified amplification product with a sequence recognised by the restriction enzyme. Therefore, the following restriction enzyme patterns are obtained:

GENOTYPE	Length of the digested DNA (in base pairs)		
wt / wt	340		
wt / mut	340	200	140
mut / mut	200		140

- The "**CYP1A1 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 340 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.

