



1. Intended Use	Code: KV0905100
The kit "MutaGEL CYP1A2" allows the detection of polymorphisms in the CYP1A2-gene encoding for the corresponding cytochrome P450 detoxifying enzyme (phase I): the two most important mutations CYP1A2*1F (C-64A) and CYP1A2*1C (G-3858A) are analysed.	

2. Introduction
Foreign chemical substances in the human organism are eliminated by own detoxifying enzymes of the body. The inducible cytochrome P450 1A2 (phase I) enzyme is mainly responsible for the activation of polycyclic aromatic hydrocarbons and plays a role in the metabolism of drugs (anti-depressiva, mexiletin, naproxen, warfarin). The C164A-mutation as well as the G3858A-mutation in the CYP1A2 gene 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. Whereas the prevalence of the C-164A mutation in Caucasians is about 15 % the G-3858 mutation is rare (< 1 %).

3. Test Principle
The kit "MutaGEL CYP1A2" contains two sets of primer pairs which each amplify a specific sequence within the human CYP1A2 gene (primer CYP2a and CYP2b). Both primer pairs were used in separated reaction tubes with the same DNA template. The present genotype of the resulting amplification products (wildtype or mutation) is analysed by restriction enzymes digestion specific for each amplicon: the mutation CYP1A2*1C forms a restriction site and mutation CYP1A2*1F reveals in the lost of a restriction site. 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)
<ul style="list-style-type: none"> ▪ primer CYP2a 1 x 30 µl solution of oligonucleotides specific for the human gene CYP1A2 (for CYP1A2*1F) ▪ primer CYP2b 1 x 30 µl solution of oligonucleotides specific for the human gene CYP1A2 (for CYP1A2*1C) ▪ primer buffer 1 x 3.0 ml buffered aqueous solution, used also as negative control ▪ dNTP mix 1 x 60 µl solution of the four dNTPs ▪ positive control DNA (2a) 1 x 30 µl aqueous solution of human DNA with the (cloned) DNA of CYP1A2 (for CYP1A2*1F) ▪ positive contro -DNA (2b) 1 x 30 µl aqueous solution of human DNA with the (cloned) DNA of CYP1A2 (for CYP1A2*1C) ▪ enzyme C2a 1 x 60 µl restriction enzyme (CYP1A2*1F) ▪ buffer for enzyme C2a 1 x 120 µl buffer for the restriction enzyme (CYP1A2*1F) ▪ enzyme C2b 1 x 60 µl restriction enzyme (CYP1A2*1C) ▪ buffer for enzyme C2b 1 x 120 µl buffer for the restriction enzyme (CYP1A2*1C)

5. Material Required but not Supplied
<p>Reagents and Instruments:</p> <ul style="list-style-type: none"> ▪ DNA extraction kit (f.e. Code.: KBR3005) ▪ Taq polymerase (5 U/ µl; f.e. Code: KDT0100) ▪ Taq reaction buffer (10 x; with MgCl₂, 15 mM) ▪ reagents for gel electrophoresis ▪ thermal cycler ▪ pipettes (0.5 -1000 µl) and sterile pipette tips (with filter) ▪ 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). Do not thaw out the content of the „CYP1A2a“ and „CYP1A2b“ positive control DNA more than two times. If necessary, make suitable aliquots. <i>Before use:</i> Spin tubes briefly before opening (contents may become dispersed during shipment).

7. Warnings and Precautions
<ul style="list-style-type: none"> ▪ For in vitro diagnostic use only. ▪ Specimens and controls should be handled as if potentially infectious. ▪ Don't use the kit after its expiration date. ▪ Set up (if possible) three separate working areas: <ol style="list-style-type: none"> 1) DNA isolation 2) Preparing amplification 3) Detection of the amplification and digestion products ▪ Use different tips and wear separate coats and gloves in each area. ▪ Use sterile plugged tips for pipetting or 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 four steps:

- 1) Sample preparation.
- 2) Separate amplification with the two primer pairs for the specific CYP1A2 mutations.
- 3) Digestion of each amplified product with a mutation specific restriction enzyme (two parallel reactions).
- 4) Detection of the amplified and digested DNA.

8. Sample Preparation

- Extract total genomic DNA f.e. from 200 µl 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).
- Each mutation (CYP1A2*1F respectively CYP1A2*1C) is analysed **separate** by parallel preparation of the amplification reactions in different reaction tubes; each with one of the both primer pairs (CYP2a or CYP2).

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	1 µl	1 µl x (N+1)
CYP2a primer respectively CYP2b primer	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 **extracted DNA** to the Master-Mix (in parallel for **reaction 2a** and **reaction 2b**)
- positive control: add 5 µl of **positive control DNA** (2a **respectively** 2b) 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 from both parallel reactions into the thermal cycler
- perform the following amplification protocol (simultaneous for **reaction 2a** and **reaction 2b** in one run):

Initial hold:	94°C for 3 min
35 cycles:	94°C for 30 sec / 60°C for 30 sec / 72°C for 1 min
Final hold:	72°C for 5 min, 4°C follow up

10. a) Digestion of the amplified DNA of reaction 2a

Prepare for each sample of **reaction 2a** 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
enzyme C2a	2 µl	2 µl x (N+1)
buffer for enzyme C2a	4 µl	4 µl x (N+1)
primer buffer	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.
- Incubate at **37°C for 3 hours** (or over night).

10. b) Digestion of the amplified DNA of reaction 2b

Prepare for each sample of **reaction 2b** 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
enzyme C2b	2 µl	2 µl x (N+1)
buffer for enzyme C2b	4 µl	4 µl x (N+1)
Primer buffer	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.
- Incubate at **37°C for 3 hours** (or over night).



11. a) Analysis of the genotype of the CYP1A2*1F allele and interpretation of the results

- Carry out a gel electrophoresis in 2 % agarose (or polyacrylamide 20 %) with about 20 µl of the amplified and digested DNA 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. 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 leads to a fragment of **920 bp** length.
- The mutation in the CYP1A2 gene (CYP1A2*1F) is identified by the lack of the restriction site for the specific restriction enzyme **C2a**. Amplified DNA from wild-type is digested by the enzyme whereas the amplified DNA from the mutated gene will not be cutted. Following restriction enzyme patterns are obtained:

GENOTYPE	Length of the digested DNA (in base pairs)
wt/ wt	709 / 211 (380 / 330)
wt/ mut	920 / 709 / 211 (380 / 330)
mut/ mut	920 (380 / 330)

- The **CYP1A2a positive control DNA (2a)** has the genotype **wt/mut**. The positive control does not show the DNA fragments 380 / 330 which are visible for the samples of extracted human DNA.
- In any case the negative controls must be negative for any amplification product.

11. b) Analysis of the genotype of the CYP1A2*1C allele and interpretation of the results

- Carry out a gel electrophoresis in 2 % agarose (or polyacrylamide 20 %) with about 20 µl of both the amplified and the digested DNA 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. 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 leads to a fragment of **568 bp** length.
- The mutation in the CYP1A2 gene (CYP1A2*1C) is identified by the lack of the restriction site for the specific restriction enzyme **C2b**. Amplified DNA from wildtype will not be digested by the enzyme whereas the amplified DNA from the mutated gene will be cutted once. Following restriction enzyme patterns are obtained:

GENOTYPE	Length of the digested DNA (in base pairs)
wt/ wt	568
wt/ mut	568 / 475 / 93
mut/ mut	475 / 93

- The **CYP1A2b positive control DNA (2b)** 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 and samples in DNA fragments of indicated length and for samples at least in the amplification products of 920 respect. 568 bp. If there are no sample DNA fragments, 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.