



<b>1. Intended Use</b>	<b>Code: KV0908100</b>
The kit "MutaGEL NAT2" allows the parallel detection of three polymorphisms (C481T, G590A and G857A) in the N-acetyltransferase 2 (NAT2) gene encoding the corresponding detoxifying enzyme (phase II).	

<b>2. Introduction</b>
Foreign chemical substances in human organism are eliminated by own detoxifying enzymes of the body. Inducible N-acetyltransferase 2 (NAT2) enzyme (phase II) transfers metabolites in water-soluble forms which can be secreted from the body. NAT2 is mainly responsible for activation of polycyclic aromatic hydrocarbons (f.e. resulting from burning processes like cigarette smoke or fried meat etc.). Individuals with NAT2 wildtype constellation are "rapid acetylators" showing normal enzyme activity. In contrast, persons with homozygous or double heterozygous mutations are "slow acetylators". Due to their restricted detoxifying capacity, these individuals have higher risk for cancer development in different organs.

<b>3. Test Principle</b>
The kit "MutaGEL NAT2" contains different sets of primer for analysis of the three mutations C481T, G590A and G857A within the human NAT2 gene. These primer sets allow a direct allele-specific amplification of the wildtype- or mutant gene constellation. Subsequent identification of present genotype is done using gel-electrophoretic methods.

<b>4. Materials Supplied (for 24 determinations)</b>
<ul style="list-style-type: none"> <li>▪ SSP-primer NAT2 wt (481C, 590G, 857G) 1 x 60 µl solution of oligonucleotides specific for NAT2 wildtype-constellation.</li> <li>▪ SSP-primer NAT2 mut (481T, 590A, 857A) 1 x 60 µl solution of oligonucleotides specific for NAT2 mutation-constellation.</li> <li>▪ primer buffer 1 x 2 ml buffered aqueous solution, used also as negative control.</li> <li>▪ dNTP-Mix 1 x 60 µl solution of the four dNTPs.</li> <li>▪ positive control DNA (PK1: wt) 1 x 50 µl aqueous solution of human DNA with (cloned) DNA of NAT2 (genotype wt/wt).</li> <li>▪ positive control DNA (PK2: mut) 1 x 50 µl aqueous solution of human DNA with (cloned) DNA of NAT2 (genotype mut/mut).</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. BLOOD MINIPREP, KBR3005)</li> <li>▪ reagents for gel electrophoresis</li> <li>▪ Taq polymerase (5 U / µl) and Taq polymerase buffer (10 x, with 15 mM MgCl<sub>2</sub>) (f.e. KDTO100)</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 "NAT2 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 three steps: <ol style="list-style-type: none"> <li>1) Sample preparation.</li> <li>2) Amplification with primers specific for wildtype- respectively mutation-constellation of NAT2 gene.</li> <li>3) Detection of the amplification products by gel electrophoresis.</li> </ol>



## 8. Sample Preparation

- Extract total genomic DNA from 200 µl of whole blood using a commercial available DNA isolation kit according to the manufacturer's manual.
- Start immediately with 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 following Master-Mix (multiply the volumes necessary for each reaction with the number **N** of reactions and add one more volume). Use for each sample two sterile PCR-micro tubes (one for **wildtype**- and **resp.** one for **mutation**- constellation)

PCR Reagents	reaction volume: 50 µl	Master-Mix volume
SSP-NAT2 primer (wt resp. mut)	2 µl	2 µl x (N+1)
Taq-polymerase buffer (10 X)	5 µl	5 µl x (N +1)
dNTP Mix	1 µl	1 µl x (N+1)
primer buffer	36.5 µl	36.5 µl x (N+1)
Taq polymerase (5U/ µl)	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 Master-Mix in both PCR-micro tubes for each one sample (**wildtype**- and **resp. mutation** tube).
- positive control: add 5 µl of **positive control DNA (PK1 resp. PK2)** to the Master-Mix in the PCR-micro tubes for positive reference.
- negative control: add 5 µl of **primer buffer** to the Master-Mix in the PCR-micro tube for negative reference.
- 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>	95°C for 3 min
<b>35 cycles:</b>	94°C for 1 min / 57°C for 1 min / 72°C for 1 min
<b>Final hold:</b>	72°C for 5 minutes, 4°C follow up

## 10. Detection of the amplified DNA

- Carry out gel electrophoresis (2 % agarose): Load for each sample about 10 µl of every both PCR products (amplificates from wildtype and mutation reaction = two lanes for one DNA-sample). Length of detected DNA fragments can be equalized with a suitable molecular weight standard. Separated DNA is colored by ethidium bromide (5 µg/ml) for 5 min and visualised under UV-light (312 nm).
- In dependence of present genotype all samples show a defined pattern of DNA-fragments. These DNA fragments correspond to the mutations as follows: 662 bp correspond to mutatio at position **481**, 549 bp correspond to mutation at position **590** and 282 bp corresponds to mutation at position **587**.

allelspecific DNA fragments	wt-tube	mut-tube	genotype
position 481: 662 bp	+		C481C = wildtype
	+	+	C481T = heterozygous
		+	T481T = homozygous
position 590: 549 bp	+		G590G = wildtype
	+	+	G590A = heterozygous
		+	A590A = homozygous
position 857: 282 bp	+		G857G = wildtype
	+	+	G857A = heterozygous
		+	A857A = homozygous

- For all three positions **positive control DNA 1** has genotype **wt/wt** and **positive control DNA 2** has genotype **mut /mut**.
- In any case the **negative control** must be negative for amplification products with indicated length.

## 12. Restrictions

The PCR results for all positive controls in DNA fragments of indicated length and for samples at least in any amplification product for wildtype- or mutation- constellation. 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.

