



## 1. Intended Use

Code: KE09001

The MutaGEL® CETP test kit allows the detection of both relevant polymorphisms (**intron 1 and 8**) in the gene of the cholesterinestertransferprotein (CETP).

## 2. Introduction

Cholesterin is an essential component of cell membranes and lipoproteins. The cholesterin metabolism regulates the individual serum lipid concentration – one of the main factors of cardiovascular diseases. Esters with unsaturated lipid acids are the mainly storage- and transport form of cholesterin. The cholesterin-estertransferprotein (CETP) controls the transport from this storage form to the liver. Polymorphisms in the CETP-Gen influence the serum lipid concentration resulting in changes of HDL concentration and LDL/HDL quotient. The biallelic polymorphism in intron 1 as well as in intron 8 of the CETP gene is associated with a lower risk for cardiovascular disease. These protective features are for the homozygous variants of intron 8 even higher than for intron 1. Both protective variants in parallel show even synergistic effects.

## 3. Principle of the Test

The kit MutaGEL® CETP contains a set of primer which amplify two specific sequences within the human CETP-gene in a specific multiplex PCR. The amplified products obtained from wild type DNA will be cut by the restriction enzyme mix in the kit, whereas the mutation constellation will not be cut. The identification of the present genotype is done by analysis of the amplification products and their cut fragments through gel electrophoresis (Dr. Essrich, Biologisches Labor, Denzlingen).

## 4. Material Supplied (24 determinations)

▪ PCR Mix (CETP)	1 x 550 µl (green)	ready to use PCR reagent ( <i>hot start</i> Taq enzyme, MgCl <sub>2</sub> , dNTP, buffer) with oligo-nucleotides specific for the human CETP gene.
▪ Positive control DNA	1 x 30 µl (red)	buffered solution with (amplified) DNA of the CETP gene.
▪ enzyme CETP 1+2	1 x 33 µl (blue)	restriction enzyme mix.
▪ buffer for enzyme CETP 1+2	1 x 320 µl (white)	buffer for restriction enzyme mix.

## 5. Materials Required but not Supplied

Reagents:

- DNA extraction kit (f.e. BLOOD MINIPREP: KBR3005)
- H<sub>2</sub>O (deionized)
- Mineral oil (optional, for thermocycler 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
- thermoblock and 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 "CETP 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. Warning 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.
- 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.
- 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 four steps:

1. Sample preparation.
2. Amplification with primers specific for CETP gene.
3. Digestion of the amplified product with a restriction enzyme.
4. Detection of the amplified and digested DNA by gel electrophoresis (size resolution).



## 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 volumes necessary for each reaction with number **N** of reactions and add 10% more volume).

PCR reagents	Reaction Volume: 25 µl	Master Mix Volume
PCR Mix (CETP)	20 µl	20 µl x N + 10 %

- For each reaction aliquot **20 µl** of the PCR Mix into a sterile microtube suitable for the thermal cycler
- Samples: add **5 µl** of the **extracted DNA** to the PCR Mix in the tube
- Positive control: add **5 µl** of the **CETP positive control DNA** to the PCR Mix in the tube
- Negative control: add **5 µl** of **H<sub>2</sub>O** to the PCR Mix in the tube
- If necessary overlay the Mix with 60 µl of mineral oil
- Transfer the microtubes into the thermal cycler
- Perform the following amplification protocol:

<b>Initial Hold:</b>	94°C for 5 min
<b>35 cycles:</b>	94°C for 30 sec / 58°C for 30 sec / 72°C for 90 sec
<b>Final Hold:</b>	72°C for 5 min, 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 10% more volume). The total volume for each Digestion Mix is **25 µl**.

Reagents for DIGESTION	Total volume for each DIGESTION: 25 µl	Volume in the Digestion-Mix
enzyme CETP 1+2	1.2 µl	1.2 µl x N + 10 %
buffer for enzyme CETP 1+2	11.3 µl	11.3 µl x N + 10 %

- aliquot **12.5 µl** of the Digestion Mix into tubes suitable for the incubator (a thermal cycler may be used for the incubation too).
- add **12.5 µl** of the amplification product to the Digestion Mix.
- transfer the tubes to the thermoblock.
- incubate at **37°C for 3 hours** (optional over night).

## 11. Detection of the Amplified/ Digested DNA and Interpretation of the Results

- Carry out gel electrophoresis in **1,5 - 2,5%** agarose (or polyacrylamide 20%) for at least **110 Vh** (f.e. 70 min at 90 volt) in 1xTBE-buffer: mix about **15 µl** of each digestion mix with **4 µl** loading buffer (f.e. KAN01070) and load the gel. The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard (f.e. KBR311005). The separated DNA is colored by ethidium bromide or SybrGreen (5 µg/ml) for 5 min and visualised under UV-light (312 nm).
- The PCR amplification leads to a fragment of **526 bp** length for the **intron 1** sequence and respectively **242 bp** length of the **intron 8** sequence.
- The presence of the **protective** gene variants (**mut**) is identified by the lack of the restriction sites in the CETP gene sequence corresponding to either intron 1 or intron 8. the amplification product obtained from the **not mutated** gene variant (**wt**) will be cut by the restriction enzyme whereas the mutated DNA sequence is not cuttable. Therefore, the following restriction enzyme patterns are obtained in relation to the present genotype:

GENOTYPE:	Intron 1	Intron 8	DNA Fragments (bp):	Enzym CETP 1	Enzym CETP 2
	mut/ mut	mut/ mut		526	242
	mut/ mut	wt/ mut		526	242 / 121
	wt/ mut	wt/ mut		526 / 354 / 172	242 / 121
	wt/ mut	mut/ mut		526 / 354 / 172	242
	wt/ wt	wt/ wt		354 / 172	121

- The **CETP positive control DNA** possesses for polymorphism in **Intron 1** genotype **wt/ mut** and for polymorphism in **Intron 8** genotype **wt/ wt**. Small amounts (about 3 %) of Intron 8- amplimer is in general restriction resistant (Drayna et al., Nucleic Acid Research, 1987) and could indicate as very weak band the position of the undigested 242 bp fragment.
- 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 indicated length. 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.