Package insert

GeneProof
Molecular diagnostics for your routine

GeneProof PAI-1 Genotyping PCR Kit

CE IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

REF	PAI1/050 50 rxn	PAI1/100 100 rxn
MasterMix PAI1	2x450 µl	4x450 µl
Positive Control PAI1 wild type	1x200 µl	1x200 µl
Positive Control PAI1 mutant	1x200 µl	1x200 µl
Positive Control PAI1 heterozygote	1x200 µl	1x200 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

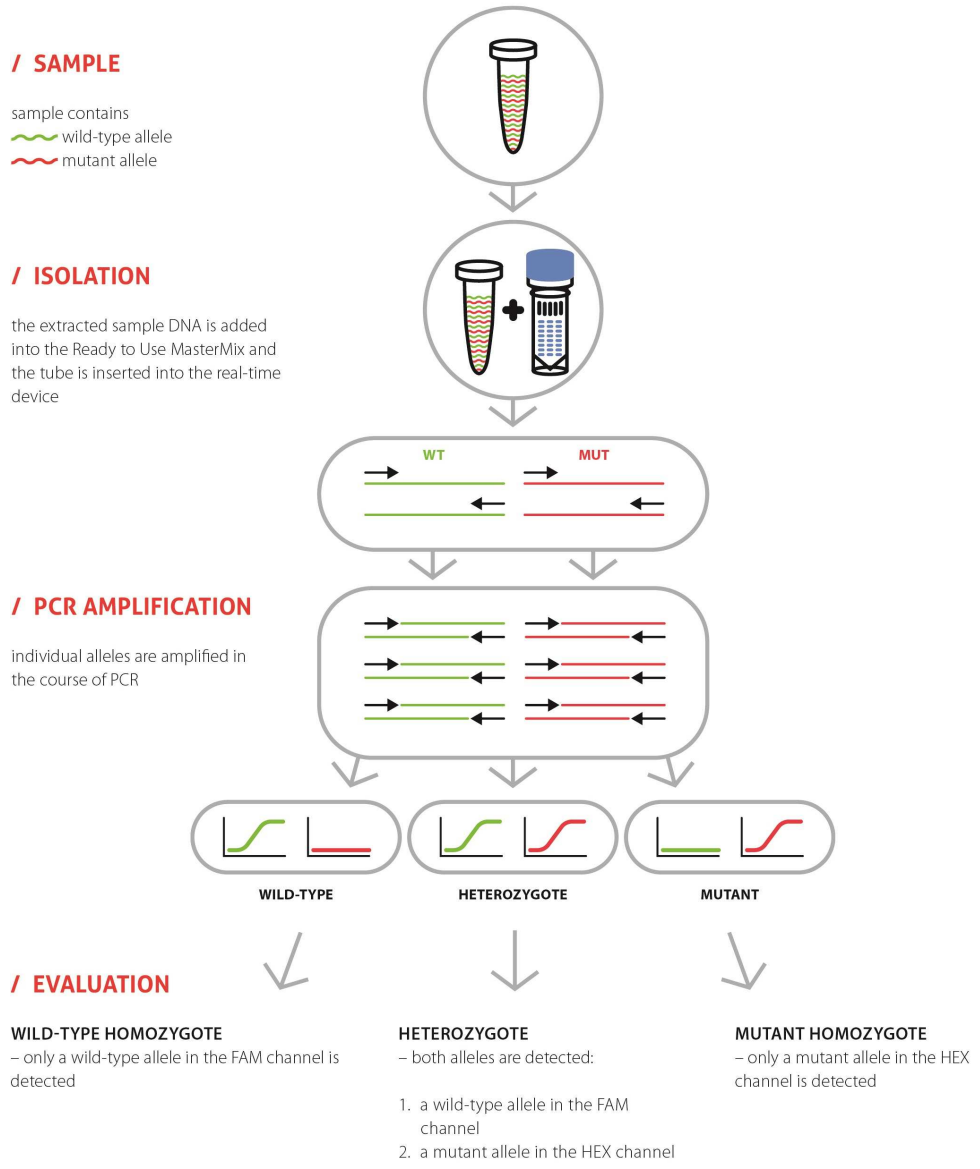
TECHNICAL SPECIFICATION

Target sequence	DNA sequence of the *PAI* gene promoter region, type 1 (Serpin E1)
Specificity	single-nucleotide insertion/deletion of guanosine in the 675 position
Qualitative detection	mutation present x not present
Evaluation	5G/5G - standard homozygote (wild type) 4G/4G - mutant homozygote 4G/5G - heterozygote
Sample types	whole blood in EDTA
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

METHOD PRINCIPLES

The PCR kit is designed to detect polymorphism in promoter of *PAI* type 1 gene by the real-time polymerase Chain Reaction (PCR) method. Method is based on amplification and detection of target sequence using allele specific fluorophore labelled probes. Target sequence is insertion-deletion polymorphism 4G/5G. Presence of wild-type allele (5G/5G) is indicated in FAM fluorescent channel and mutant allele (4G/4G) in HEX fluorescent channel. In case of heterozygous genotype (4G/5G) signal is detected in both channels. Detection kit contains Ready to Use MasterMix and utilizes "hot start"technology, minimizing non-specific reactions and assuring maximum sensitivity. Kit is designed for *in vitro* diagnostic.

GENETIC DIAGNOSTIC TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Sampling does not require any special preparation of the patient. Usually 3 ml of peripheral blood are sampled into a tube with EDTA. It is possible to keep the blood in a refrigerator at the temperature between +2 °C and +8 °C for 3 days; for long-term storage keep the samples at the temperature between -85 °C and -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleid Acid Extraction System

PCR SETUP

1. Add 18 µl of MasterMix into PCR tubes.

2. Add 2 µl of the DNA sample or 2 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 20 µl.
It is necessary to keep all components at +2°C to +8°C during the PCR preparation.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data collection	Cycles
1. Hold	95 °C	10 min		1
2. PCR	95 °C	10 s	FAM+HEX	40
	64 °C	20 s		
	72 °C	20 s		

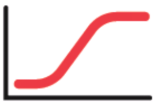
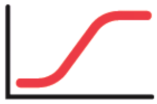
VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

Applied Biosystems 7300/7500 Real-Time PCR System
Dx/CFX96™/CFX Connect™ Real-Time PCR Detection System
LightCycler ® 480
LineGene 9600
Rotor-Gene 3000, Rotor-Gene 6000
SLAN® Real-Time PCR System
StepOne™ Real-Time PCR System

GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.

CLINICAL SAMPLES ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation
		Valid	5G/5G – standard homozygote (wild type)
		Valid	4G/4G – mutant homozygote
		Valid	4G/5G – heterozygote
		Invalid	

WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

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