Package insert

GeneProof
Molecular diagnostics for your routine

GeneProof MTHFR A1298C 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	M1298/050 50 rxn	M1298/100 100 rxn
MasterMix MTHFR A1298C	2x450 µl	4x450 µl
Positive Control MTHFR A1298C wild type	1x50 µl	1x50 µl
Positive Control MTHFR A1298C mutant	1x50 µl	1x50 µl
Positive Control MTHFR A1298C heterozygote	1x50 µl	1x50 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.

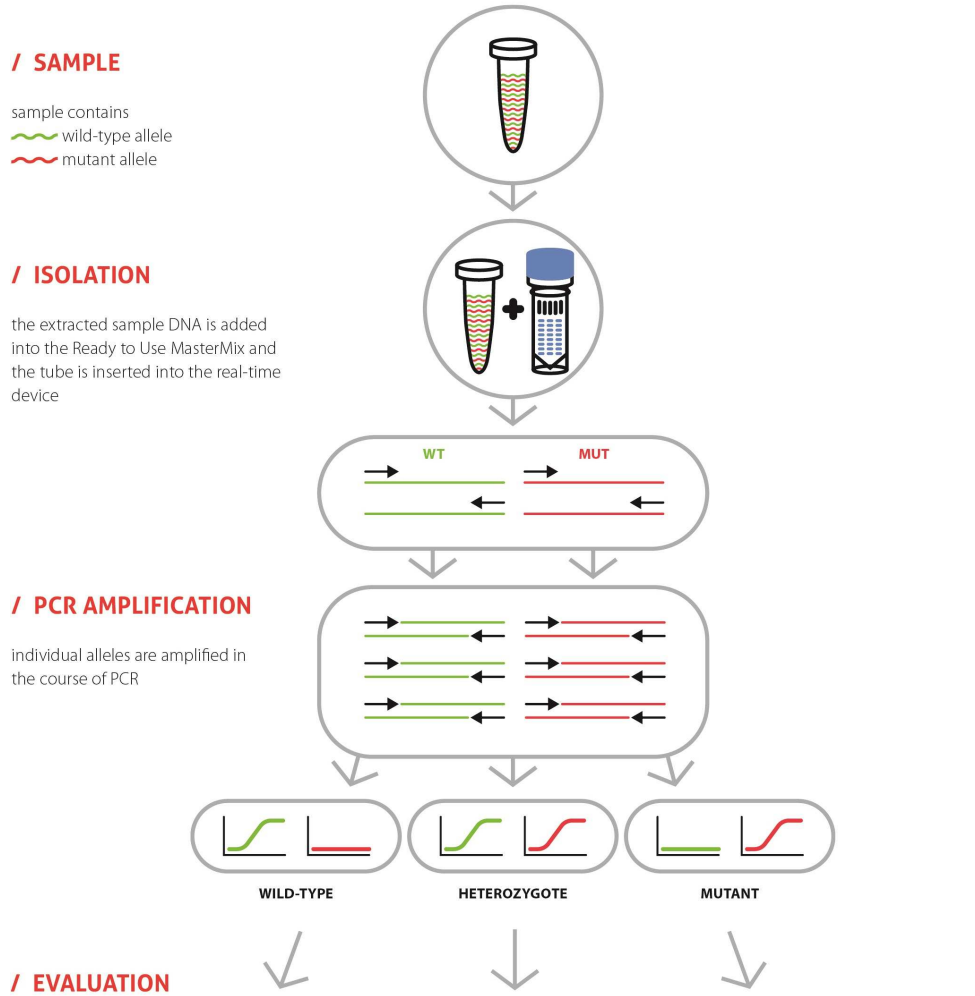
TECHNICAL SPECIFICATION

Target sequence	DNA sequence of a gene encoding methylenetetrahydrofolate reductase (MTHFR)
Specificity	A1298C single-nucleotide mutation (Glu 429 Ala)
Qualitative detection	mutation present x not present
Evaluation	A/A - standard homozygote (wild type) C/C - mutant homozygote A/C - heterozygote
Sample types	whole blood EDTA
Quality Control	regularly tested by Instand e.V. External Quality Assessment Panel

METHOD PRINCIPLES

The PCR kit is designed to detect A1298C mutation in gene for methylenetetrahydrofolate reductase (MTHFR) 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 single nucleotide replacement of adenine for cytosine in site 1298 (A1298C). Presence of wild-type allele (A1298A) is indicated in FAM fluorescent channel and mutant allele (C1298C) in HEX fluorescent channel. In case of heterozygous genotype (A1298C) 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		

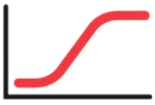
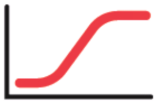
VALIDATED INSTRUMENTS

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 ® 2.0, 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	A/A – standard homozygote (wild type)
		Valid	C/C - mutant homozygote
		Valid	A/C – 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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