

GeneProof MTHFR A1298C PCR Kit



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	M1298/025 25 rxn	M1298/050 50 rxn	M1298/100 100 rxn
MasterMix MTHFR A1298C	1x450 µl	2x450 µl	4x450 µl
Positive Control MTHFR A1298C wt	1x50 µl	1x50 µl	1x50 µl
Positive Control MTHFR A1298C mut	1x50 µl	1x50 µl	1x50 µl
Positive Control MTHFR A1298C het	1x50 µl	1x50 µl	1x50 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 15 repeated freezing/thawing cycles.

TECHNICAL SPECIFICATION

Target Sequence	A1298C mutation in the gene for methylenetetrahydrofolate reductase (MTHFR)
Sensitivity	reaches up to 2 ng/µl
Diagnostic Specificity	100 %
Diagnostic Sensitivity	100 %
Evaluation	A/A - standard homozygote (wild type) C/C - mutant homozygote A/C - heterozygote
Validated Specimen	Whole blood
Quality Control	In accordance with ISO 13485, each lot of GeneProof PCR Kit is tested against predetermined specifications to ensure consistent product quality.
External Quality Assessment	Regularly tested by Instand e.V. External Quality Assessment

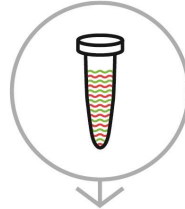
METHOD PRINCIPLES

The PCR kit is designed to detect A1298C mutation in the gene for methylenetetrahydrofolate reductase (MTHFR) by the real-time Polymerase Chain Reaction (PCR). The method is based on amplification and detection of the target sequence using allele specific fluorophore labelled probes. The target sequence is the single nucleotide adenine/cytosine polymorphism in site 1298 (A1298C). Presence of wild-type allele (A1298A) is detected in the FAM fluorescent channel and mutant allele (C1298C) in the HEX fluorescent channel. In case of heterozygous genotype (A1298C) a signal is detected in both channels. Detection kit contains Ready to Use MasterMix and takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Kit is designed for *in vitro* diagnostic.

GENETIC DIAGNOSTIC TECHNOLOGY

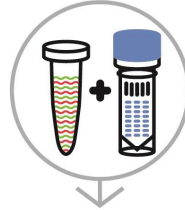
/ SAMPLE

sample contains
— wild-type allele
— mutant allele



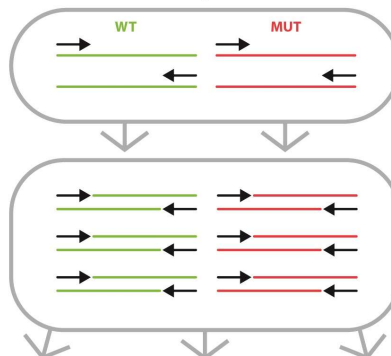
/ ISOLATION

the extracted sample DNA is added into the Ready to Use MasterMix and the tube is inserted into the real-time device



/ PCR AMPLIFICATION

individual alleles are amplified in the course of PCR



/ EVALUATION

WILD-TYPE HOMOZYGOTE

– only a wild-type allele in the FAM channel is detected

HETEROZYGOTE

– both alleles are detected:

1. a wild-type allele in the FAM channel
2. a mutant allele in the HEX channel

MUTANT HOMOZYGOTE

– only a mutant allele in the HEX channel is detected

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 -20 ± 5 °C.

NUCLEIC ACID PURIFICATION

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

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleic Acid Extraction System

PCR SETUP

1. Add 18 µl of MasterMix into PCR tubes.
2. Add 2 µl of the isolated nucleic acid 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
Hold	95 °C	10 min		1
	95 °C	10 s		
PCR	64 °C	20 s	FAM+HEX	40
	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:

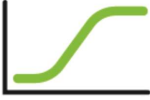



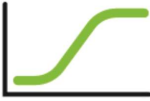
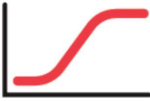


croBEE Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 2.0 / 480
LineGene 9600 Plus
Rotor-Gene 3000 / 6000
SLAN® Real-Time PCR System

Required Detection Channels FAM, HEX

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.

Customer care and technical support

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Orders

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