

GeneProof®

MTHFR C677T

PCR Kit



in vitro Diagnostics

The kit is designed for professional use in specialized clinical and research laboratories.

Principle of the method

This kit is designed for detection of the C677T mutation in the 5, 10-Methylenetetrahydrofolate reductase (*MTHFR*) gene using the real-time PCR method. This method consists in the PCR amplification of the DNA sequence bearing the C677T mutation and hybridisation of the amplified sequence with the fluorophore-marked probes for the healthy allele C677C and for the mutant allele T677T of the *MTHFR* gene.

Kit composition

	Cat. No. M677/RG/025 25 reactions	Cat. No. M677/RG/050 50 reactions	Cat. No. M677/RG/100 100 reactions
MasterMix <i>MTHFR CT</i>	1 x 450 µl	2 x 450 µl	4 x 450 µl
Positive Control <i>CT wt</i>	1 x 50 µl	1 x 50 µl	1 x 50 µl
Positive Control <i>CT mut</i>	1 x 50 µl	1 x 50 µl	1 x 50 µl
Positive Control <i>CT het</i>	1 x 50 µl	1 x 50 µl	1 x 50 µl

Storage of the kit

The recommended storage temperature is –20 to –80°C. Under this optimal temperature range, the kit is stable for 6 months from the date of manufacturing. Repeated thawing and freezing of the kit may result in lower detection quality. Therefore, the manufacturer strongly recommends to divide the MasterMix into smaller working aliquots and keep them under the temperature –20 °C until used. Positive controls can be stored at 4°C.

Operating instructions

DNA Isolation

The manufacturer recommends following commercial DNA isolation kits: PathogenFree DNA Isolation Kit (GeneProof); Arrow Blood DNA Kit (NorDiag).

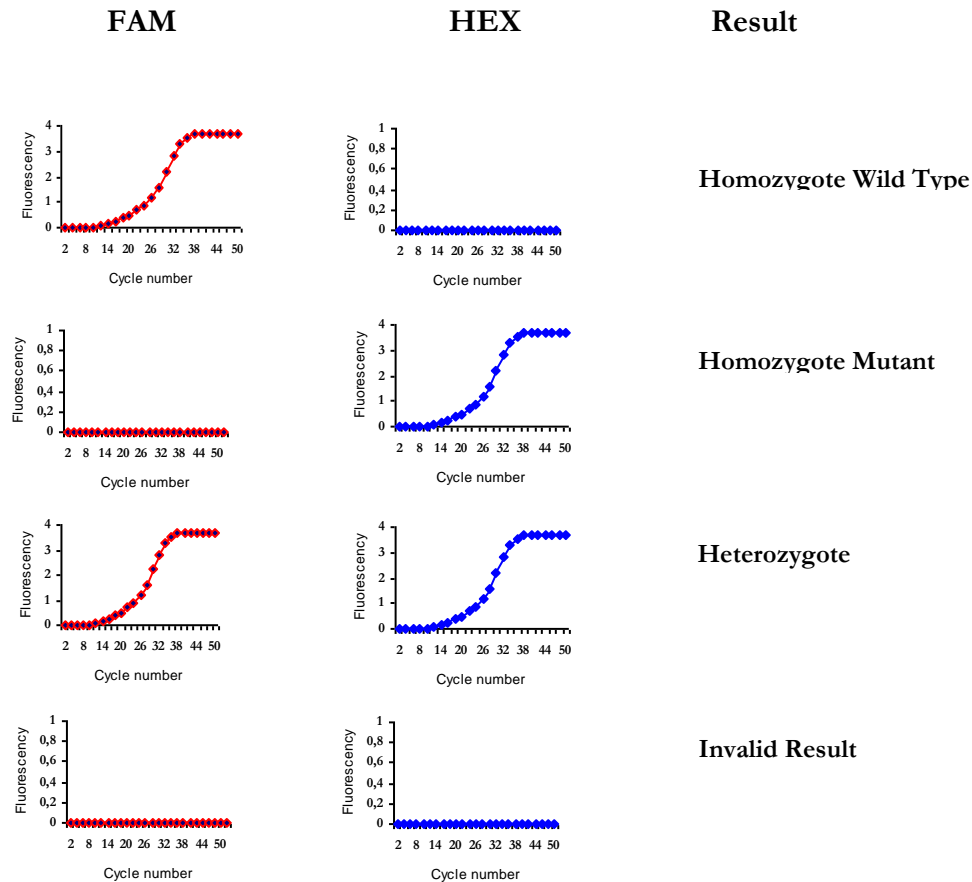
Preparation of the PCR reactions

1. Add **18 µl of Master Mix** into the PCR tubes.
2. Add **2 µl of the DNA template** or **2 µl of Positive Control** into the individual PCR tubes.
During PCR preparation, all ingredients should be kept on ice (temperature 0 – 6 °C).
3. Insert the tubes with prepared PCR reactions into the thermocycler and close the lid.

Programming of the PCR profile

		Acquisition mode	
Hold		95°C 10 min	Not Acquiring
		95°C 10 sec	Not Acquiring
Cycling	40 cycles	64°C 20 sec	Acquiring FAM and HEX fluorescent signal
		72°C 20 sec	Not Acquiring

Result analysis



GeneProof PCR kits are designed to be performed on real-time instruments of different manufacturers. With following real-time instruments Factor II Prothrombin PCR Kit was validated:

Rotor-Gene™ 3000 (Corbett Life Science)
Rotor-Gene™ 6000 (Corbett Life Science)
LightCycler® 2.0 (Roche)
LightCycler® 480 System (Roche)
SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)

Ask distributor of the kits for detailed manuals for the particular real-time devices or download them from the www.geneproof.com
If you want use kit with other instrument mentioned above, contact please our Product Support Department at: support@geneproof.com

For information about using the PCR kits and assessing their results see the detailed manuals supplied by the manufacturer with the individual Real Time devices.

Warning:

- The kit has been manufactured in harmony with the EC Directive 98/79/EC as an *in vitro* medical diagnostic device.
- 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.
- 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.