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

<table>
<thead>
<tr>
<th>REF</th>
<th>M677/025</th>
<th>M677/050</th>
<th>M677/100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 rxn</td>
<td>50 rxn</td>
<td>100 rxn</td>
</tr>
</tbody>
</table>

MasterMix
MTHFR C677T
- 1x450 µl
- 2x450 µl
- 4x450 µl

Positive Control
MTHFR C677T
- wt
  - 1x50 µl
  - 1x50 µl
  - 1x50 µl

- mut
  - 1x50 µl
  - 1x50 µl
  - 1x50 µl

- 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
C677T mutation in the gene for methylenetetrahydrofolate reductase (MTHFR)

Sensitivity
Reaches up to 2 ng/µl

Diagnostic Specificity
100 %

Diagnostic Sensitivity
100 %

Evaluation
- C/C - standard homozygote (wild type)
- T/T - mutant homozygote
- C/T - 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 panels
The PCR kit is designed to detect C677T 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 cytosine/thymine polymorphism in site 677 (C677T). Presence of wild-type allele (C677C) is detected in the FAM fluorescent channel and mutant allele (T677T) in the HEX fluorescent channel. In case of heterozygous genotype (C677T) 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.
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 below -20 ± 5 °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 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

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Data Collection</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold</td>
<td>95 °C</td>
<td>10 min</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>PCR</td>
<td>95 °C</td>
<td>10 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>64 °C</td>
<td>20 s</td>
<td>FAM+HEX</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>72 °C</td>
<td>20 s</td>
<td></td>
<td></td>
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</table>

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
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

<table>
<thead>
<tr>
<th>Channel FAM</th>
<th>Channel HEX</th>
<th>Result</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td>[ ]</td>
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<td>Valid</td>
<td><strong>C/C – standard homozygote (wild type)</strong></td>
</tr>
<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>Valid</td>
<td><strong>T/T - mutant homozygote</strong></td>
</tr>
<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>Valid</td>
<td><strong>C/T – heterozygote</strong></td>
</tr>
<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>Invalid</td>
<td></td>
</tr>
</tbody>
</table>

**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**

Tel.: +420543211679  
Fax: +420516770824  
email: support@geneproof.com

**Orders**

Tel.: +420543211679  
Fax: +420516770824  
email: sales@geneproof.com