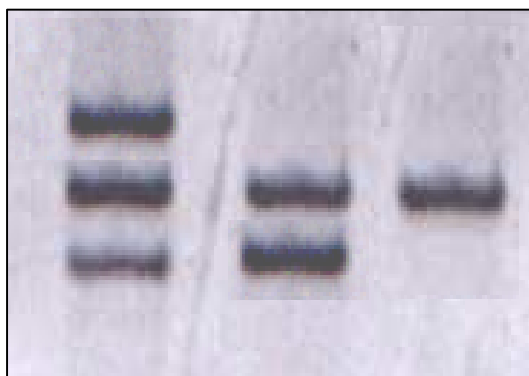


 ATGen SISTEMAS MOLECULARES	Code: KIT-005	Ver: 1
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MTHFR C677T

Detection system of the C677T mutation in the human
Methylen-tetrahydrofolate reductase gene



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The purchase of this product does not provide a license to carry out patented applications.

Kit utility

This kit analyzes the presence of the C677T polymorphism in exon 4 of the methylen tetrahydrofolate reductase (MTHFR) gene, which is responsible for the substitution of an alanine for a valine in the 222nd amino acid of the protein.

Principle of the Assay


The detection of the C677T mutation in the MTHFR gene is performed by PCR amplification followed by restriction enzyme digestion. The A1298C alteration is subsequently evidenced with the same strategy.

The analysis is based on PCR amplification of the MTHFR gene fragment in which the presence or absence of a base substitution is later detected by RFLP. The restriction enzyme recognizes two restriction sites in the amplified fragment of a mutated individual. When a normal allele is amplified, one of these restriction sites disappears while the other one remains. This strategy has the advantage of providing a digestion control site in the amplified sequence.

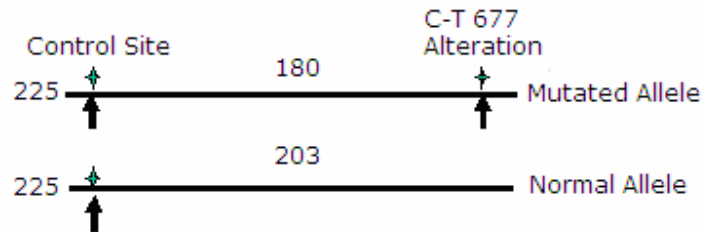
After the digestion it is possible to define a mutated band and a normal band in the electrophoretic pattern.

Introduction

The MTHFR gene is located in the 1p36.3 region of the short arm in chromosome 1. The 5-10-methyltetrahydrofolate reductase enzyme catalyzes the reduction of 5-10 methyltetrahydrofolate into 5-methyltetrahydrofolate, which is the primary form of serum folate, a co-substrate for the re-methylation of homocysteine into methionin. The substitution of a cytosine for a thymine in the 677th position of the MTHFR gene (C677T) produces a thermolabile version of the enzyme which has lower activity levels, thus affecting the serum levels of homocysteine. A1298C is another polymorphism which also reduces the enzymatic activity.

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Experimental strategy



Kit Presentation

Color that identifies the kit: Blue

The ATGen MTHFR C677T Kit includes:

- 1 MTHFR C677T Reaction Mix tube (blue solution).
- 1 MTHFR C677T Restriction Enzyme tube.
- 1 MTHFR C677T Control DNA containing heterozygote control DNA (once defrosted it is recommended to keep it at 4 °C).
- 1 MTHFR C677T Taq DNA polymerase tube.
- 1 MTHFR C677T Molecular Weight tube. It contains the bands corresponding to the amplification product and the two possible bands that result from digestion. This tube should be kept in the post-amplification zone if possible.

The kit must be kept at -20 °C.

Our kits are available in two sizes: 20 and 50 reactions

Necessary materials not provided with the test

- DNA-free PCR tubes
- Suitable Pipettes
- Filtered pipette tips
- Gloves and robe
- Thermocycler
- Cuvette for vertical electrophoresis
- Acrylamide, electrophoresis buffer and loading buffer
- Gel coloring system with silver nitrate.
- Vortex
- Bio-hazard disposable container

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Precautions

1. Only for in-vitro use.
2. All samples, reagents and controls should be considered potentially infectious.
3. Do not use after the expiration date indicated on the package.



Storage and Stability

The kit must be stored at -20°C in order to assure its optimal performance through the expiration date indicated on the package.

Specimen Characteristics

The sample must be a DNA solution with a 150-200 ng / µl concentration, apt for PCR amplification.

ATGen recommends obtaining the DNA from blood specimens by using ADN Facil kit.

Protocol

Defrost the reaction mix and shake it vigorously by vortexing.
If possible, perform all manipulations in cold room.

Preparation of the amplification mix:

- 1. Add 18 µl of reaction mix per sample to be tested.
- 2. Add to the reaction mix, 1 µl of DNA Taq polymerase per sample to be tested.
- 3. Homogenize by moderate vortexing or pipetting.

It is recommended to prepare a single amplification mix containing the necessary quantities of reaction mix and DNA Taq polymerase, according to the number of samples to be analyzed.


Note: It is suggested to add an extra volume (aprox. 10%) of each reagent to the amplification mix in order to compensate pipetting errors.

It is necessary to add two reactions, one for the positive control and one for the negative control.

Amplification:

- 4. Aliquot the amplification mix, dispensing 18 µl in properly labeled PCR tubes.
- 5. Add 2 µl of sample to each tube.

The samples must contain between 30 and 50 ng of DNA (We recommend to use ATGen's ADN Facil Kit for the DNA extraction).

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- 6. Add 2 μ l of MTHFR C677T control DNA into the positive control tube and 2 μ l of the water previously used to dissolve the sample DNA in the negative control tube.
- 7. Run the program for MTHFR C677T.

MTHFR C677T program: 30 cycles at 94 °C/0:30 ´, 59 °C/0:30 ´, 72 °C/0:30 ´; an initial denaturing step: 3 minutes at 94 °C and a final extension step: 3 minutes at 72 °C.

- 8. Place the tubes into the thermocycler when it reaches 94°C.

Once the program has ended and in the case you are not going to immediately perform the next step, keep the tubes at 4°C until the digestion stage.

Optionally, the amplification can be tested by electrophoresis by loading 5 μ l of the reaction product and using a 6% acrylamide gel or a 2 % agarose gel.


The expected size of the amplification product is 225 pb.

Digestion:

- 9. Once the program has ended allow the temperature to drop until the tubes reach room temperature and then add 0.5 μ l of restriction enzyme to every amplification tube.
- 10. Homogenize using the pipette.
- 11. Incubate for 1:30 hrs at 37 °C (it is also possible to incubate overnight) and then 10 minutes at 65 °C

Obtention of test results

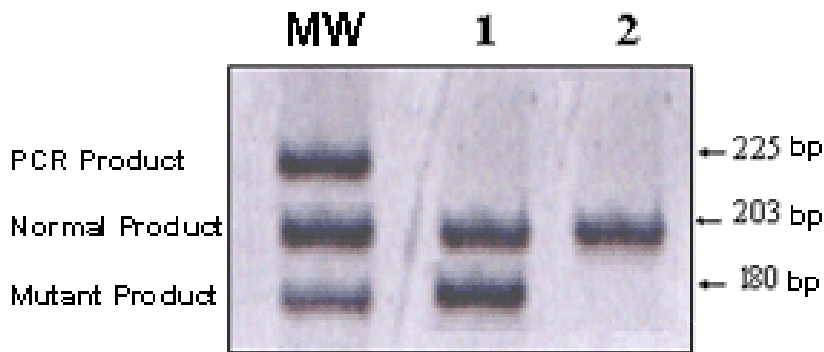
- Prepare the samples using the indicated quantities of an adequate buffer (p.e. glycerol 30% p/c, xilencianol blue 0.25% p/v, bromophenol blue 0.25% p/v).
- Load 5 μ l of each digested amplification product and 5 μ l of the MTHFR C677T molecular weight marker in a 6% acrylamide gel or 20 μ l of each one in a 2% agarose gel.
- Migrate the bromophenol blue (of the loading buffer): 8 cm in acrylamide or 3.5 cm in agarose.
- Use silver nitrate for staining the acrylamide gel or use uv light for viewing the agarose gel.

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Interpretation of test results

Electrophoretic pattern	Result
203 bp	Normal Homozygote
203 + 180 bp	Heterozygote
180 bp	Mutated Homocygote

Example:



6% Acrylamide gel showing the following results:

Lane 1: Heterozygote mutated Individual (The control DNA must show this pattern)

Lane 2: Normal individual

MW: Molecular Weight marker including all possible products

Band of 203 bp = "normal band"

Band of 180 bp = "mutated band"

The 225 bp band must not be present if the digestion was complete.

Bibliography

1. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 2000;151:862-77.
2. OMIM, 607093 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE; MTHFR