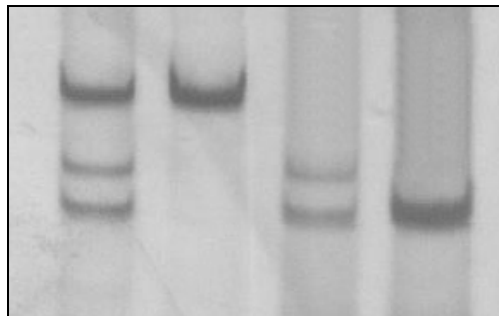




MTHFR A1298C

Detection system of the A1298C mutation in the human
Methylen-tetrahydrofolate reductase gene



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 ATGen <small>SCIENTIFIC SOLUTIONS</small>	Code: IDK-006	Ver: 1
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Kit utility

This kit analyzes the presence of the A1298C polymorphism in the methylen-tetrahydrofolate reductase (MTHFR) gene.

Principle of the Assay

The detection of the A1298C mutation in the MTHFR gene is performed by PCR amplification followed by restriction enzyme digestion (RFLP).

There are three possible test results:

Homozygote AA: when the A1298C mutation cannot be detected in neither of the two alleles of the gene.

Heterozygote AC: when the A1298C mutation is detected in one of the alleles of the gene.

Homozygote CC: when both alleles present the mutation.

Introduction

5,10- Methylene-tetrahydrofolate reductase (MTHFR) is an enzyme that catalyses the reduction of the 5,10-methylene-tetrahydrofolate into 5-methylene-THF, the primary form of serum folate, which acts as co-substrate for the re-methylation of homocysteine into methionine.

The MTHFR A1298C polymorphism produces the substitution of a glutamate for an alanine in the protein, thus reducing its enzymatic activity.

Another polymorphism, identified as C677T, produces a thermolabile version of the enzyme which also has a reduced activity, affecting the homocysteine serum level.

Kit presentation

Color that identifies the kit: Pink

The ATGen MTHFR A1298C Kit includes:

- 1 MTHFR A1298C Reaction Mix tube (blue solution).
- 1 MTHFR A1298C Restriction Enzyme tube.
- 1 MTHFR A1298C control DNA containing heterozygote DNA (once defrosted it is recommended to keep it at 4 °C).
- 1 MTHFR A1298C Taq DNA polymerase tube.
- 1 MTHFR A1298C Molecular Weight tube. It contains three bands corresponding to the amplification product and the two possible digestion bands. 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

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 50-100 ng / µl concentration, apt for PCR amplification.

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

Protocol

Pre-amplification zone:

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 50 and 100 ng of DNA (We recommend to use ATGen's ADN Facil Kit for DNA extraction).

- 6. Add 2 µl of MTHFR A1298C control DNA into the positive control tube and 2 µl of the water previously used to dissolve the sample DNA in the negative control tube.
- 7. Run the program for MTHFR A1298C.

Program MTHFR A1298C: 30 cycles at 94 °C/0:30'; 56 °C/0:30'; 72 °C/0:30' an initial denaturing step: 5 minutes at 94 °C and a final extension step: 5 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 µl of the reaction product and using a 6% acrylamide gel or a 2 % agarose gel.

The expected size of the amplification product is 216 bp.

Digestion:

- 8. Once the program has ended, allow the temperature to drop until the tubes reach room temperature and then add 1 µl of restriction enzyme to each amplification tube.
- 9. Homogenize using the pipette.
- 10. Incubate for 3 hrs at 37 °C (it is possible to incubate overnight).

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 µl of each digested amplification product and 5 µl of the MTHFR A1298C molecular weight marker in a 6% acrylamide gel or 15 µl of each in a 2% agarose gel pre-stained with ethidium bromure (0.5µg/ml).
- Migrate the bromophenol blue (of the loading buffer) until the end in acrylamide or 3cm in agarose.

- Use silver nitrate for staining the acrylamide gel or use uv light for viewing agarose gels.

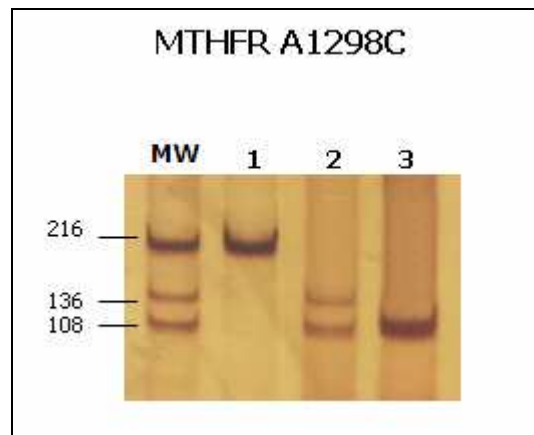
Interpretation of test results

	Homozygote AA	Heterozygote AC	Homozygote CC
MTHFR A1298C	108 bp	136 + 108 bp	136 bp

Note:

The 225 bp band must not be present if the digestion was complete. If this band is present (in top of the marker) it means partial digestion. In this case, add another 1µl of restriction enzyme to the partially digested products and incubate at 37°C for an additional two hour period.

Example:



6% Acrylamide gel stained with silver nitrate, showing two of the possible results:

- 1: PCR product (216 pb)
 - 2: AC heterozygote individual (A1298C DNA control must show this result)
 - 3: AA homozygote individual.
- MW: A1298C molecular weight marker with the amplification band and the possible digestion bands.

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

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