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MTHFR C677T HRM kit

MBK0024
50 Reactions (42 tests)
Store at -20°C

Intended use

For identification of MTHFR C677T polymorphism by High Resolution Melt (HRM).

Introduction

5,10-methylenetetrahydrofolate reductase enzyme, encoded by MTHFR gene, catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The MTHFR C677T polymorphism results in the amino acid change from Ala to Val. The Val allele is associated with decreased enzyme activity and low tetrahydrofolate concentrations. The reduced enzyme activity is involved in homocysteinemie, an important risk factor for thrombotic disease.
The presence of MTHFR C677T polymorphism can be investigated in the polyabortivity screening and it is associated with outcome of 5 FU treatment in Coloretal Cancer chemotherapy [Victor Cohen et al. 2003].
The MTHFR C677T HRM kit represents an alternative PCR-based approach for the detection of the cited polymorphism, suitable for a relatively inexpensive screening of several samples, reducing the turnaround time and the workload. Focused on the melting curve analysis obtained with a high resolution melt (HRM) system, it can be applied to the analysis and discrimination of more than one amplicon with the same reaction in the same run; today HRM is the most cost-efficient method for SNP identification. This kit provides genotype controls to compare results from unknown samples.

Product description

The MTHFR C677T HRM kit is an easy-to-use master mix dedicated for the use with the Rotor-gene 6000 instrument (Corbett Research) and Rotor –Gene Q (QIAGEN). The kit contains reagents, enzyme and genotype controls for the detection of MTHFR C677T SNP located in the MTHFR gene by HRM. The kit performs 42 tests in two separated runs.

Kit contents

1 x MTHFR C677T 2X master mix (650 µl)
1 x DNA polymerase (10 µl)
2 X MTHFR C677T WT CONTROL (10 µl)
2 X MTHFR C677T MUT CONTROL(10 µl)
2 X MTHFR C677T HET CONTROL(10 µl)

Storage

The product should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, the kit is stable up to the expiry date indicated in the attached label.
Note: The product is guaranteed for two thawing steps. For an intermittently use we suggest to subdivide MTHFR C667T Master mix in small aliquots.

Precautions

The user should always pay attention to:

- use pipette tips with aerosol-preventive filters, deionized DNA-free water and gloves;
- store positive material (plasmidic DNA controls) separately from all other reagents and, if possible, add it to the reaction mix in a separated space;
- do not use the same precision pipettes for reaction mix and DNA;
- thaw all components samples at room temperature before starting an assay;
- when thawed, mix the components and centrifuge briefly.
- protect the MTHFR C667T Master mix from light

Procedure

PREPARING HRM GENOTYPIC CONTROLS
Genotype controls must be prepared by 1:100 dilution of stock solution in PCR –grade water (not provided).
Genotype controls are provided to characterize the HRM curves of unknown amplicons.

PCR SETUP
Total volume per reaction is 25 µl.

Before each use, thaw all reagents completely, mix and centrifuge.
Pipet mastermix and the Taq polymerase in the quantity needed for the planned reactions into a 1.5 ml reaction tube and mix as indicated in table 1 .

To detect potential contaminations include for each experiments at least 1 negative template control (NTC), containing all reaction components except for DNA sample.
Moreover include provided positive controls for each assay as references for genotype assignment.

Table 1

	1 reaction	50 reaction
MTHFR C677T 2X PCR Mastermix	12.5 µl	625 µl
DNA Polymerase (5U/µl)	0.125 µl	6.25 µl
H_2O	10.375	518.75
Total volume	23 µl	1150 µl

Aliquot 23 µl of master mix into each PCR reaction tube before adding 2 µl sample DNA*, or 2 µl water (negative control) or 2 µl positive controls (MTHFR C677T WT-MUT-HET) for each SNP genotype of interest .

*Employ 10 ng DNA/reaction (2 µl of a 5ng/µl concentrated DNA).
(DNA quality is a fundamental factor for HRM genotyping: kit has been optimized with DNA extracted by **DNeasy Blood & Tissue Kit** -QIAGEN and **Blood genomic DNA isolation mini Kit -** NORGEN. Alternative isolation methods have to be validated from the user. In any case we suggest to elute purified DNA in water.)

After pipetting the negative control and the samples, the tubes must be sealed in order to avoid cross-contamination during the addition of positive control.

THERMAL PROFILE
HRM analysis setting is a prerequisite for accurate results. For details, please refer to the manual provided with your HRM real-time PCR instrument.
Program the HRM instrument according to the operator's manual and table 2

Table 2

STEP	TEMPERATURE	TIME	CICLES
Initial denaturation	95°C	10 min	1X
Denaturation	95°C	10 sec	40X
Annealing / Extension	59°C	40 sec	
Acquire on the GREEN Channel in the Annealing / Extension step			
High Resolution melting	Ramp 78°-89°C	Rise temp 0.2°C/step; wait for 90 sec pre-melt conditions; wait for 2 sec for each step afterwards; For optimal acquisition of fluorescence data, set the gain to 70% of saturation in the highest fluorescent signal.	
Acquire on the HRM channel			

ANALYSIS SETUP

For HRM analysis set confidence level at 90%.

Due to the comparison between controls and unknown samples HRM profiles, the software instrument performs an automatic genotyping showing a confidence level for each sample.
Results with a confidence level lower than 90% are defined "variation" and have to be reject.

Troubleshooting

No signal, poor Rn value (PCR or HRM) or signal detected late in PCR	Pipetting error or missing reagent	Check the storage conditions of the reagents, Repeat the assay.
	Problems with starting template DNA	Check the concentration, storage conditions, and quality of the template and control DNA. Efficient removal of PCR inhibitors is essential for optimal results; purify nucleic acids from your sample using an appropriate purification method. Insufficient or degraded template DNA, increase the amount of template DNA if possible.

References

Victor Cohen et al. Methylenetetrahydrofolate Reductase Polymorphism in Advanced Colorectal Cancer: A Novel Genomic Predictor of Clinical Response to Fluoropyrimidine-based Chemotherapy1. *Clinical Cancer Research 1611*.

Fernández-Peralta AM et al. Association of polymorphisms MTHFR C677T and A1298C with risk of colorectal cancer, genetic and epigenetic characteristic of tumors, and response to chemotherapy. *Int J Colorectal Dis. 2010*

Paola Terrazzi et al. Homocysteine, MTHFR C677T gene polymorphism, folic acid and vitamin B 12 in patients with retinal vein occlusion. *Thrombosis Journal* 2005.

Images
Images

C-C

C-T

T-T

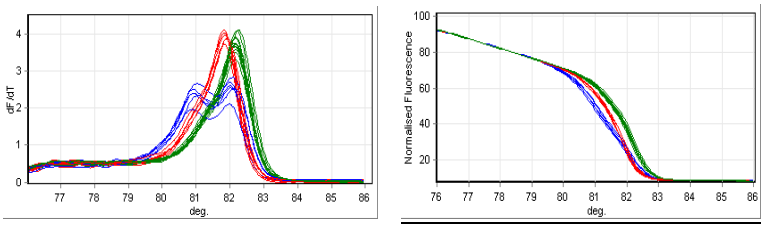

Fig. 1: Graphical visualizations for the identification of the MTHFR C677T polymorphisms.
A) Melting curves show heteroduplexes formation in the C-T population (in blue) and temperature shift between C-C and T-T genotypes (green and red curves, respectively).
B) Normalized melting curves of the three genotypes.