

MTHFR C677T (Methylentetrahydrofolat-Reductase) ToolSet™ for LightCycler™

Lyophilized ToolSet for PCR using the LightCycler™ Instrument. Licensed by Roche Diagnostics GmbH

Order#: MTHFR 677 - 16

1 ToolSet for 16 reactions

Store at 4°C, protected from light.
Exposure to light may especially damage the Oligotool™ tube (vial with red cap).

For use with LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102)

1. ToolSet contents

| Vial | Label | Content | Quantity |
|---------------------|------------------|---|---|
| | | | MTHFR 677 - 16 |
| 1, Red cap | OligoTool | - lyophilized oligos for PCR - contains mutation detection and anchor probe, primers | For 16 tests Dissolved: 50 µL |
| 2, Green cap | Control | - lyophilized heterozygous DNA | Dissolved: 20 µL |
| 3, Blue cap | Solvent | - to dissolve OligoTool / Control | 1000 µL of Solvent |

Additional equipment and reagents required but not supplied :
LightCycler-DNA Master Hybridization Probes, 10 x conc. Cat.No.: 2 015 102, including 25mM MgCl₂; LightCycler instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description

This ToolSet is specifically designed for genotyping the C677T polymorphism in the Methylentetrahydrofolat-reductase (MTHFR) gene by LightCycler PCR with Melting Curve Analysis. The primer pair and fluorescent detection and anchor probes have been optimized for specific amplification of a 166 bp segment containing the potentially mutated site and for optimal genotype discrimination.

Control material

Heterozygous control DNA, lyophilized.

Storage of ToolSet and Solutions

Store at +4°C when lyophilized, protected from light.
The unopened lyophilized ToolSet is stable at +4°C for 12 months from date of manufacture if protected from light. When dissolved store at +4°C for a maximum of 4 weeks, or at -20°C for longer periods (months), protected from light. Avoid freezing and thawing.

3. Preparation for LightCycler PCR

Toolset preparation **Dissolve** the content of the **OligoTool** tube (Red Cap) with **50 µl of Solvent**.
Dissolve the content of the **Control** tube (Green Cap) with **20 µl of Solvent**.

1. Before opening tubes, centrifuge them quickly.
2. Add Solvent into OligoTool tube and Control tube as above.
3. Recap tubes, vortex gently.
4. Before opening tubes, centrifuge them quickly.
5. Proceed to Reaction Mix preparation.

Primers ? You don't have to add primers.

Probes ? You don't have to add probes.

Reaction Mix Preparation For 1 (One) reaction, prepare the Reaction Mix as shown in the following table :

| Reagent | µL |
|-------------------------------------|------------------|
| OligoTool MTHFR 677 -16, dissolved | 2.8 |
| Solvent MTHFR 677 -16 | 9.6 |
| MgCl ₂ 25 mM | 1.6 (final 3 mM) |
| Master Hybridization Probes 10x | 2 |
| Total Reaction Mix | 16 |
| + Your DNA or Control MTHFR 677 -16 | 4 |
| Grand Total | 20 |

Use Master Hybridization Probes 10x and MgCl₂ 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102, including 25mM MgCl₂).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control Always run a positive control with the samples. Use the dissolved heterozygous Control MTHFR 677 - 16 DNA (Green Cap).

Negative control Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Solvent (Blue Cap).

Extraction of genomic DNA You can use different Kits for DNA isolation, either with a manual method or with an automated system. The elution buffers should be salt-free. Example : Roche High Pure PCR Template Preparation Kit (Cat.No. 1 796 828)

Application The **MTHFR C677T** ToolSet™ for LightCycler™ allows detection of the **C677T** single nucleotide polymorphism in the MTHFR gene, causing to an Ala-to-Val exchange, with the Val-containing enzyme being thermolabile and less active. **MTHFR 677 TT** has been associated with hyperhomocysteinaemia in end-stage renal disease, neural tube defects (spina bifida, nonsyndromic cleft lip and Down's syndrome), development of cancers and leukaemia and toxicity of anti-folate drugs like Methotrexate. Also, the homozygous **MTHFR 677 TT** variant enhances the risk of deep venous thrombosis associated with the **Factor V Leiden** mutation similar to the **PAI-1 4G5G polymorphism**.

Note : This ToolSet employs the same temperature-time protocol as the PAI-1 4G/5G ToolSet and can be used in the same LightCycler run.

Note : This ToolSet was developed for use in life science research only.

4. LightCycler Settings and Experimental Protocol

Denaturation

| Cycle Program Data | Value |
|-----------------------------------|------------------|
| Cycles | 1 |
| Analysis Mode | None |
| Temperature Targets | Segment 1 |
| Target Temperature (°C) | 95 |
| Incubation time (s) | 60 |
| Temperature Transition Rate (°/s) | 20 |
| Secondary Target Temperature (°C) | 0 |
| Step Size (°C) | 0 |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

Amplification

| Cycle Program Data | Value | | |
|-----------------------------------|------------------|------------------|------------------|
| Cycles | 50 | | |
| Analysis Mode | None | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 3 |
| Target Temperature (°C) | 95 | 57 | 72 |
| Incubation time (s) | 1 | 5 | 3 |
| Temperature Transition Rate (°/s) | 20 | 20 | 3 |
| Secondary Target Temperature (°C) | 0 | 0 | 0 |
| Step Size (°C) | 0 | 0 | 0 |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | Single | None |

Melting Curve Analysis

| Cycle Program Data | Value | | |
|-----------------------------------|------------------|------------------|------------------|
| Cycles | 1 | | |
| Analysis Mode | Melting Curves | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 3 |
| Target Temperature (°C) | 95 | 40 | 80 |
| Incubation time (s) | 60 | 60 | 0 |
| Temperature Transition Rate (°/s) | 20 | 20 | 0.1 |
| Secondary Target Temperature (°C) | 0 | 0 | 0 |
| Step Size (°C) | 0 | 0 | 0 |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | None | Continuous |

Cooling

| Cycle Program Data | Value |
|-----------------------------------|------------------|
| Cycles | 1 |
| Analysis Mode | None |
| Temperature Targets | Segment 1 |
| Target Temperature (°C) | 40 |
| Incubation time (s) | 30 |
| Temperature Transition Rate (°/s) | 20 |
| Secondary Target Temperature (°C) | 0 |
| Step Size (°C) | 0 |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

LC Program Version and Fluorescence Display Mode

Developed with LC Program Version 3.3. Use F2/F1 or preferably F2 with colour compensation; with gains F1=1; F2=15. For LC Program Versions 3.5 and higher : use automatic gain control.

5. Typical results

Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples. The melting peaks allow discrimination between the possible genotypes of the C677T polymorphism in the MTHFR gene. Figure 1 shows a typical result obtained with the MTHFR C677T ToolSet™ for LightCycler™ :

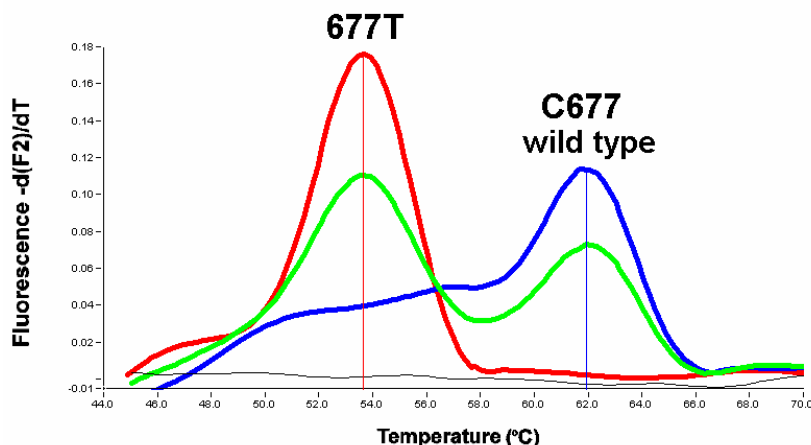


Figure 1 : Melting curve analysis of possible genotypes of the C677T polymorphism in the MTHFR gene.

BLUE : Homozygote for **C677 (wild type)** , **RED** : Homozygote for **677T** , **BLACK** : No DNA Control.

GREEN : Heterozygote Control contained in the ToolSet, Control **MTHFR C677T HET**.

Blue Cursor : $T_m = 61.9$ °C , **Red Cursor** : $T_m = 53.6$ °C.

Conditions : LC program version 3.5 with automatic gain setting, Color compensation and Digital Filter enabled, Calculation Method : Polynomial, Degrees to average : 10.

Note : The values for the respective melting temperatures may vary for +/- 2.5 °C between different experiments. The Delta T between the melting peaks for different genotypes may vary +/- 1.0 °C. The MTHFR C677T ToolSet™ has been developed for and validated with the LightCycler™ and its original accessory materials and reagents. Performance of the ToolSet with other instruments, accessories and reagents has not been validated by ratiogen.

7. Notices to Purchaser : Licenses and Trademarks, Prohibition of Resale

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