

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

## Application on LightCycler 480

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 Fast Start DNA Master HybProbes, 10 x conc. (Roche Cat.No.: 03003248001)** and **LightCycler® 480 Multiwell Plates 96, white (Roche Cat.No.: 04729692001)**

### 1. ToolSet contents

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

Additional equipment and reagents required but not supplied :  
LightCycler® Fast Start DNA Master HybProbes, 10 x conc.Cat.No.: 03003248001, including 25mM MgCl<sub>2</sub>;  
LightCycler® 480 instrument, LightCycler® 480 Multiwell Plates 96 white, DNA extraction materials

### 2. Introduction

#### 2.1. Product overview

**ToolSet description** This ToolSet is specifically designed for genotyping the C677T polymorphism in the Methylentetrahydrofolatreductase (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 times.

### 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 <sub>2</sub> 25 mM	1.6 (final 3 mM)
Fast Start DNA Master HybProbes, 10x	2
Total Reaction Mix	16
+ Your DNA or Control MTHFR 677 -16	4
Grand Total	20

Use Fast Start DNA Master Hybridization Probes 10x and MgCl<sub>2</sub> 25 mM from Roche LightCycler Fast Start DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 03003248001, including 25mM MgCl<sub>2</sub>).  
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 was developed for use in life science research only.

#### 4. LightCycler 480 Settings and Experimental Protocol

For use with LC 480 Program Version 1.5 series. Detection : Dynamic Red 640 (498-640 nm).

##### Fast Start Enzyme Activation and DNA Denaturation

Cycle Program Data	Value
Cycles	1
Analysis Mode	None
Temperature Targets	<b>Segment 1</b>
Target Temperature (°C)	<b>95</b>
Incubation time (s) <b>Hold</b>	<b>600</b>
Temperature Transition Rate (°C/s) <b>Ramp Rate</b>	<b>4.4</b>
<b>Acquisitions (per °C)</b>	---
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

##### Amplification

Cycle Program Data	Value		
Cycles	<b>40</b>		
Analysis Mode	Quantification		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	<b>95</b>	<b>60</b>	<b>72</b>
Incubation time (s) <b>Hold</b>	<b>5</b>	<b>10</b>	<b>15</b>
Temperature Transition Rate (°/s) <b>Ramp Rate</b>	<b>4.4</b>	<b>2.2</b>	<b>4.4</b>
<b>Acquisitions (per °C)</b>	---	---	---
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	<b>Single</b>	None

##### Melting Curve Analysis

Cycle Program Data	Value		
Cycles	<b>1</b>		
Analysis Mode	Melting Curves		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	<b>95</b>	<b>40</b>	<b>85</b>
Incubation time (s) <b>Hold</b>	<b>30</b>	<b>120</b>	---
Temperature Transition Rate (°/s) <b>Ramp Rate</b>	<b>4.4</b>	<b>1.5</b>	<b>0.29</b>
<b>Acquisitions (per °C)</b>	---	---	<b>2</b>
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	None	<b>Continuous</b>

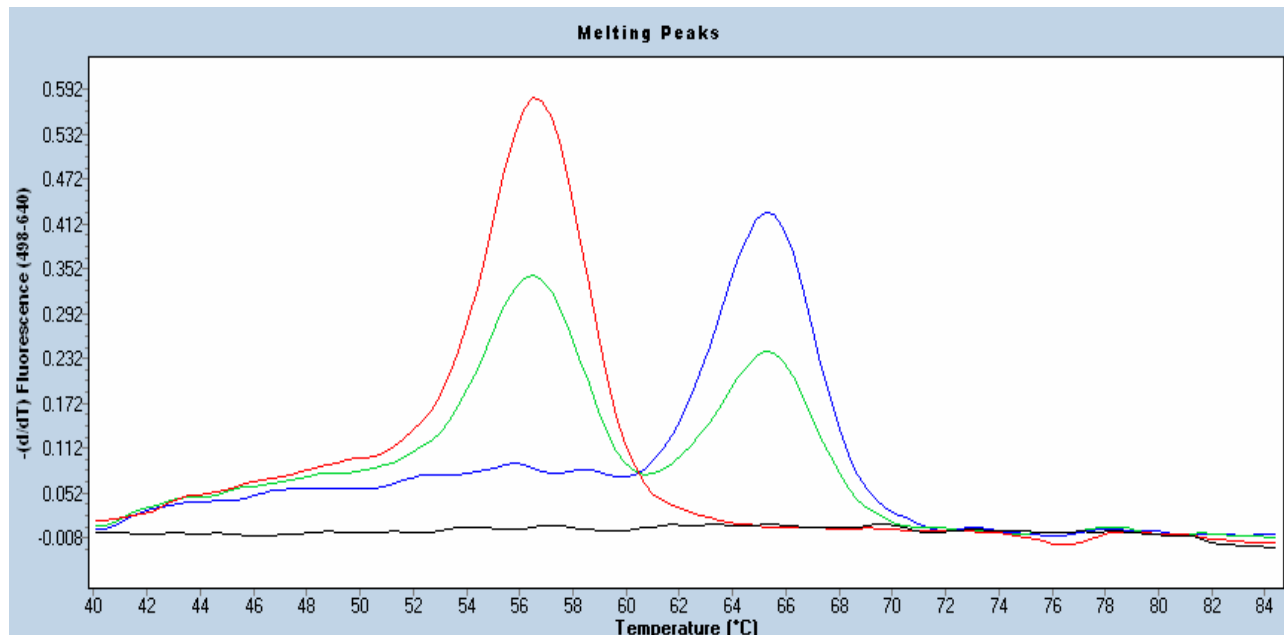
##### Cooling

Cycle Program Data	Value
Cycles	<b>1</b>
Analysis Mode	None
Temperature Targets	<b>Segment 1</b>
Target Temperature (°C)	<b>40</b>
Incubation time (s) <b>Hold</b>	<b>30</b>
Temperature Transition Rate (°/s) <b>Ramp Rate</b>	<b>1.5</b>
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

## 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 **CC677** ( $T_m = 65.3$  °C, wild type)
- RED** : Homozygote for **677TT** ( $T_m = 56.5$  °C),
- GREEN** : Heterozygote Control contained in the ToolSet, Control **MTHFR C677T HET**.
- BLACK** : No DNA Control

Conditions : LC 480 program version 1.5, No Color compensation,

**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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