

## CYP2C9 A1075C ToolSet™ for LightCycler™ (CYP2C9\*1 / CYP2C9\*3 Allele, Oral Anticoagulation, Pharmacogenetics)

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

**Order#: CYP2C9 1075 - 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 FastStart DNA Master HybProbe**, 10 x conc. (Roche Cat.No.: 03003248001)

### 1. ToolSet contents

| Vial                | Label            | Content  | Quantity                         |
|---------------------|------------------|--|----------------------------------|
|                     |                  |  | <b>CYP2C9 1075 - 16</b>          |
| <b>1, Red cap</b>   | <b>OligoTool</b> | - lyophilized oligos for PCR<br>- contains mutation detection<br>and anchor probe, primers | For 16 tests<br>Dissolved: 50 µL |
| <b>2, Green cap</b> | <b>Control</b>   | - lyophilized heterozygote DNA   | Dissolved:<br>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 FastStart DNA Master Hybridization Probes, 10 x conc. Cat.No.: 03003248001, including 25mM MgCl<sub>2</sub>; LightCycler instrument, LightCycler capillaries, DNA extraction materials

### 2. Introduction

#### 2.1. Product overview

**ToolSet description** This ToolSet is specifically designed for genotyping the A1075C mutation in the CYP2C9 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 180 bp segment containing the potentially mutated site and optimal genotype discrimination.

**Control material** Heterozygote 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 ml of Solvent**.  
**Dissolve** the content of the **Control** tube (Green Cap) with **20 ml 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 CYP2C9 1075 -16, dissolved      | 2.8              |
| Solvent CYP2C9 1075 -16                   | 8.8              |
| MgCl <sub>2</sub> 25 mM                   | 2.4 (final 4 mM) |
| <b>FastStart DNA Master HybProbe, 10x</b> | 2                |
| Total Reaction Mix                        | 16               |
| + Your DNA or Control CYP2C9 1075 -16     | 4                |
| Grand Total                               | 20               |

Use LightCycler FastStart DNA Master Hybridization Probes 10x and MgCl<sub>2</sub> 25 mM from Roche LightCycler FastStart 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 CYP2C9 A1075C heterozygous Control 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 **CYP2C9 gene** encodes a major isoform of cytochrome P450 responsible for oxidation of a wide array of drugs including coumarins commonly employed for oral anticoagulation.  
 The **CYP2C9 A1075C** ToolSet™ for LightCycler™ allows detection of the **A→C** mutation at position 1075 in the CYP2C9 gene causing an Ile359Leu amino acid exchange.  
 The CYP2C9 1075C mutation (CYP2C9\*3 allele) is associated with impaired CYP2C9-mediated metabolism and enhanced sensitivity to coumarins.

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

#### 4. LightCycler Settings and Experimental Protocol

##### Denaturation and FastStart Activation

| Cycle Program Data                | Value            |
|-----------------------------------|------------------|
| Cycles                            | 1                |
| Analysis Mode                     | None             |
| Temperature Targets               | <b>Segment 1</b> |
| Target Temperature (°C)           | 95               |
| Incubation time (s)               | <b>600</b>       |
| 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                            | <b>38</b>  |
| Analysis Mode                     | None   |
| Temperature Targets               | <b>Segment 1</b> <b>Segment 2</b> <b>Segment 3</b>     |
| Target Temperature (°C)           | 95 <b>59</b> 72  |
| Incubation time (s)               | 5                        5                        5    |
| Temperature Transition Rate (°/s) | 20                      20                      20     |
| 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               | <b>Segment 1</b> <b>Segment 2</b> <b>Segment 3</b>         |
| Target Temperature (°C)           | 95                      45                      75         |
| Incubation time (s)               | 60                      60                      0          |
| Temperature Transition Rate (°/s) | 20                      20                      0.2        |
| 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               | <b>Segment 1</b> |
| 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             |

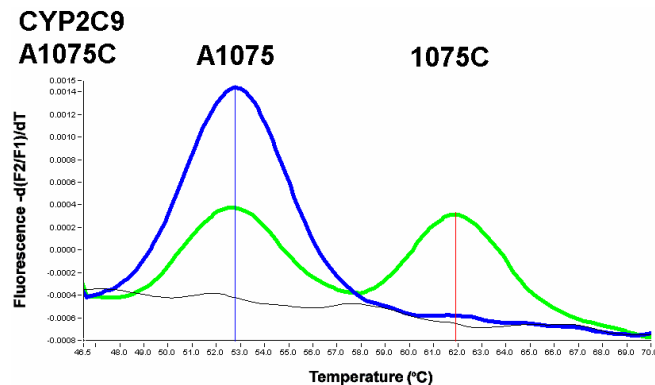
##### LC Program Version and Fluorescence Display Mode

Developed with LC Program Version 3.5 and automatic gain control.  
For fluorescence display use F2/F1, or F2 with colour compensation.

## 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 at the **A1075C** mutation site in the **CYP2C9** gene. Figure 1 shows a typical result obtained with the **CYP2C9 A1075C ToolSet™** for LightCycler™ :



**Figure 1 : Melting curve analysis of genotypes at position 1075 of the human CYP2C9 gene**

**BLUE :** Homozygote **AA 1075** (wild type)  
**GREEN :** Heterozygote **A1075C** (Control DNA contained in the ToolSet)

**BLACK :** No DNA Control.

**Blue Cursor :**  $T_m = 52.7\text{ }^\circ\text{C}$  ; **Red Cursor :**  $T_m = 61.9\text{ }^\circ\text{C}$

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation, Digital Filter enabled, Degrees to average : 11.0. Calculation Method : Polynomial.

**Note :** The values for the respective melting temperatures may vary for  $\pm 2.5\text{ }^\circ\text{C}$  between different experiments. The Delta T between the melting peaks for different genotypes may vary  $\pm 1.0\text{ }^\circ\text{C}$ . The CYP2C9 A1075C 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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