

# HFE C282Y ToolSet™ for LightCycler™

## Application on LightCycler 480

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

**Order#: HFE 282 - 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>HFE 282 - 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** The ToolSet is specifically adapted for genotyping HFE Amino Acid position 282 by LightCycler PCR with Melting Curve Analysis. Fluorescent detection and anchor probes and the primer pair have been optimized for specific amplification of targets and 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 HFE 282-16 dissolved	2.8
Solvent HFE 282-16	9.6
MgCl <sub>2</sub> 25 mM	1.6
Fast Start DNA Master HybProbes, 10x	2
Total Reaction Mix	16
+ Your DNA or Control HFE 282-16	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 HFE 282-16 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 HFE C282Y ToolSet™ for LightCycler™ allows the detection of the single point mutation resulting in the C282Y variant of the HFE gene which is found in 50-90 % of cases of hereditary haemochromatosis.

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) with Colour Compensation

##### 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>45</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>55</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>43</b>	<b>80</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>

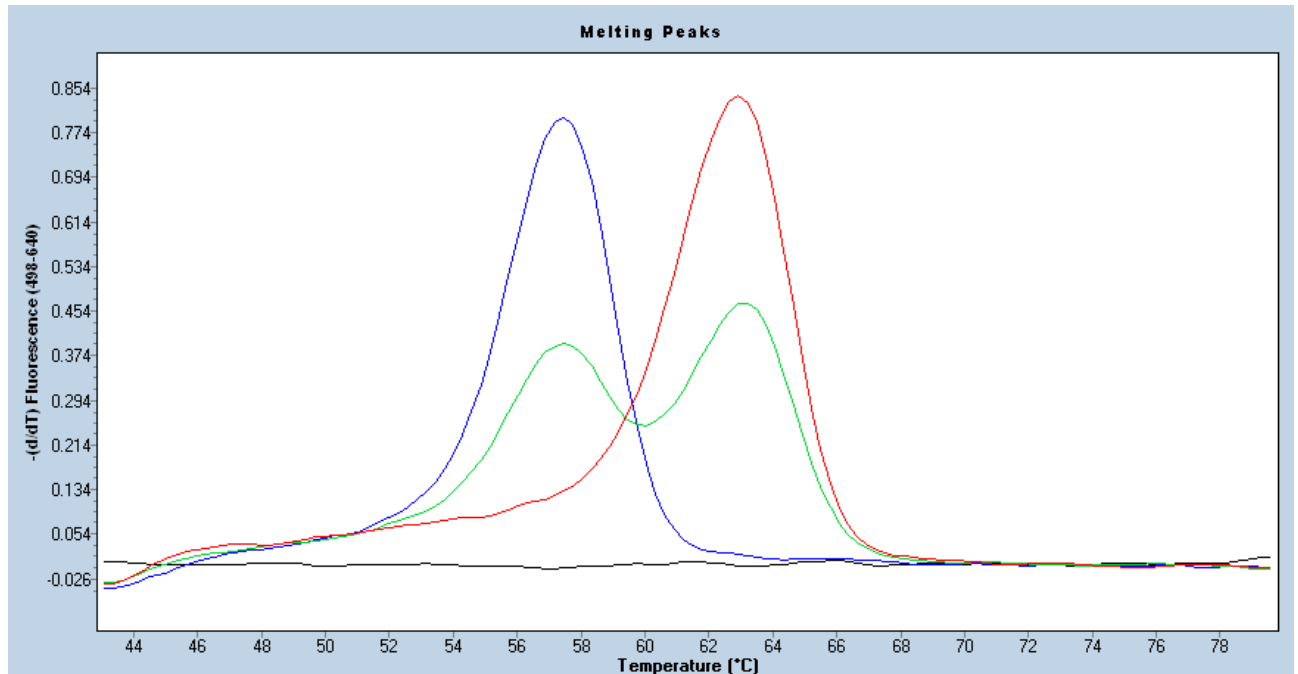
##### Cooling

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 homozygous (wild type or mutant) and the heterozygous samples. Figure 1 shows a typical result obtained with the HFE C282Y ToolSet™ for LightCycler™ :



**Figure 1 : Melting curve analysis of the three possible genotypes of the HFE sequence at AA 282.**

**BLUE :** Homozygote **CC 282** ( $T_m = 57\text{ }^{\circ}\text{C}$ , wild type)

**RED :** Homozygote **282 YY** ( $T_m = 63\text{ }^{\circ}\text{C}$ )

**GREEN :** Heterozygote Control contained in the ToolSet, Control **HFE C282Y HET**

**BLACK :** No DNA Control

Conditions : LC 480 program version 1.5, with Colour 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 HFE C282Y 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.

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

The purchase of this product does not convey any right for its use in clinical diagnostics. No license to use the PCR process is conveyed expressly or by implication by purchase of this product. LightCycler and High Pure are trademarks of members of the Roche group. Resale of ratiogen's products is expressly prohibited.

### How to contact ratiogen

E-mail [info@ratiogen.com](mailto:info@ratiogen.com)

Internet <http://www.ratiogen.com>