

HFE H63D+S65C ToolSet™ for LightCycler™

Application on LightCycler 480

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

Order#: HFE 6365 - 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
			HFE 6365 - 16
1, Red cap	OligoTool	- lyophilized oligos for PCR - contains mutation detection and anchor probe, primers	For 16 tests Dissolved: 50 µL
2 A–B , Green caps	2 Controls	- lyophilized heterozygous DNA	Dissolved: 20 µL each
3, Blue cap	Solvent	- 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 MgCl₂;
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 positions 63 and 65 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 2 Heterozygous control DNA's : **A = H63D, B = S65C** ; 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 HFE 6365 -16 dissolved	2.8
Solvent HFE 6365 -16	10
MgCl ₂ 25 mM	1.2 (2.5 mM final)
Fast Start DNA Master HybProbes, 10x	2
Total Reaction Mix	16
+ Your DNA or Control HFE 6365 -16	4
Grand Total	20

Use Fast Start DNA Master Hybridization Probes 10x and MgCl₂ 25 mM from Roche LightCycler Fast Start DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 03003248001, including 25mM MgCl₂).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control Always run a positive control with the samples. To save reagents, You may use routinely only the dissolved heterozygous **Control HFE 6365 -16 DNA A = H63D** and save the heterozygous Control HFE 6365 -16 DNA **B = S65C** for confirmation runs with samples suspected to carry the rare S65C mutation (both with Green Caps).

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 H63D+S65C ToolSet™ for LightCycler™ allows the detection of the single point mutations resulting in the H63D and S65C variants of the HFE gene found in a fraction of cases of hereditary haemochromatosis.

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

Note !: This ToolSet employs the same LC program as the HFE C282Y ToolSet.

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	Segment 1
Target Temperature (°C)	95
Incubation time (s) Hold	600
Temperature Transition Rate (°C/s) Ramp Rate	4.4
Acquisitions (per °C)	---
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

Amplification

Cycle Program Data	Value		
Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	55	72
Incubation time (s) Hold	5	10	15
Temperature Transition Rate (°/s) Ramp Rate	4.4	2.2	4.4
Acquisitions (per °C)	---	---	---
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	43	80
Incubation time (s) Hold	30	120	---
Temperature Transition Rate (°/s) Ramp Rate	4.4	1.5	0.29
Acquisitions (per °C)	---	---	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	Segment 1
Target Temperature (°C)	40
Incubation time (s) Hold	30
Temperature Transition Rate (°/s) Ramp Rate	1.5
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 at AA positions 63 and 65 of the HFE gene product. Figure 1 shows a typical result obtained with the HFE H63D+S65C ToolSet™ for LightCycler™ :

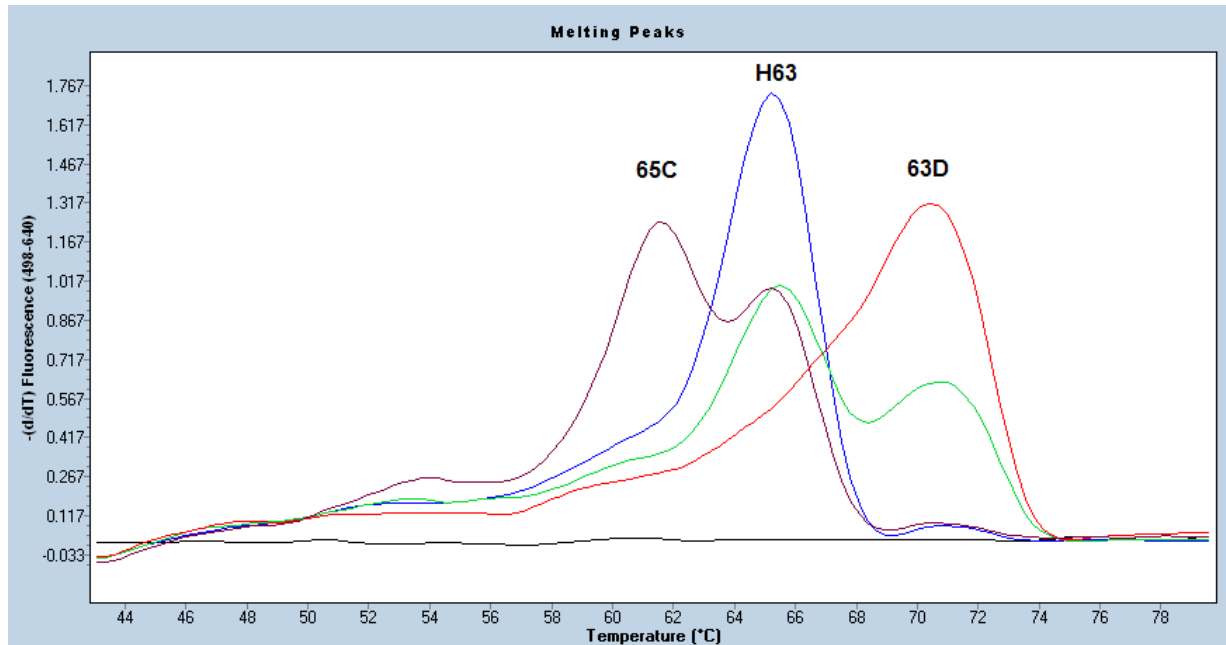


Figure 1 : Melting curve analysis of possible genotypes of the HFE sequence at AA's 63 and 65.

- BLUE :** Homozygote **HH 63** ($T_m = 65.3\text{ }^{\circ}\text{C}$, wild type)
- RED :** Homozygote **63 DD** ($T_m = 70.5\text{ }^{\circ}\text{C}$, Mutant)
- GREEN:** Heterozygote **Control A = H63D**, contained in the ToolSet
- PINK:** Heterozygote **Control B = S65C** ($T_m = 61.5\text{ }^{\circ}\text{C}$ for 65C Mutant), contained in the ToolSet
- Black : Negative Control without DNA.

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 H63D+S65C 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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