

Factor V Leiden ToolSet™ for LightCycler™

Application on LightCycler 480

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: FVL - 16

1 Kit 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
			FVL - 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® 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

Kit description

The **FV Leiden ToolSet™** is specifically designed for genotyping the Human Factor V gene for presence of the Factor V Leiden (G1691A, R506Q, rs 6025) mutation by Real Time PCR with Melting Curve Analysis. Primer pair and fluorescent detection probes have been optimized for specific amplification of a 222 bp segment containing the potentially mutated site 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 24 months from date of manufacture if protected from light. When dissolved store at +4°C for a maximum of 1 week, or at -20°C for longer periods (months), protected from light. Avoid freezing and thawing > 10 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.

4.

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

Reagent	µL
OligoTool FVL, dissolved	2.8
Solvent FVL	8.8
MgCl ₂ 25 mM	2.4 (final 4 mM)
FastStart DNA Master HybProbe, 10x	2
Total Reaction Mix	16
+ Your DNA or Control FVL	4
Grand Total	20

Use FastStart DNA Master HybProbe 10x and MgCl₂ 25 mM from Roche (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. Use the dissolved heterozygous Control FVL 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).

DNA Extraction The Kit has been evaluated with the Qiagen Blood Mini Kit.

Application The **FV Leiden** ToolSet™ for LightCycler™ has been specifically designed for the detection of the Factor V Leiden mutation in the Human Factor V gene. The Factor V Leiden mutation is associated with venous thrombosis.

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

Note : This ToolSet employs the same LightCycler Time-Temperature protocol (next page) as the Factor II G20210A ToolSet and can be used in the same run.

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	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	35		
Analysis Mode	Quantification		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	60	72
Incubation time (s) Hold	5	10	10
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	40	85
Incubation time (s) Hold	60	60	---
Temperature Transition Rate (°/s) Ramp Rate	4.4	2	0.14
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 samples. Melting peaks allow discrimination between the genotypes of the human Factor V gene at nt position 1691, i.e. wild type, heterozygous and homozygous FV Leiden. **Figure 1 shows a typical result obtained with the FV Leiden ToolSet™ :**

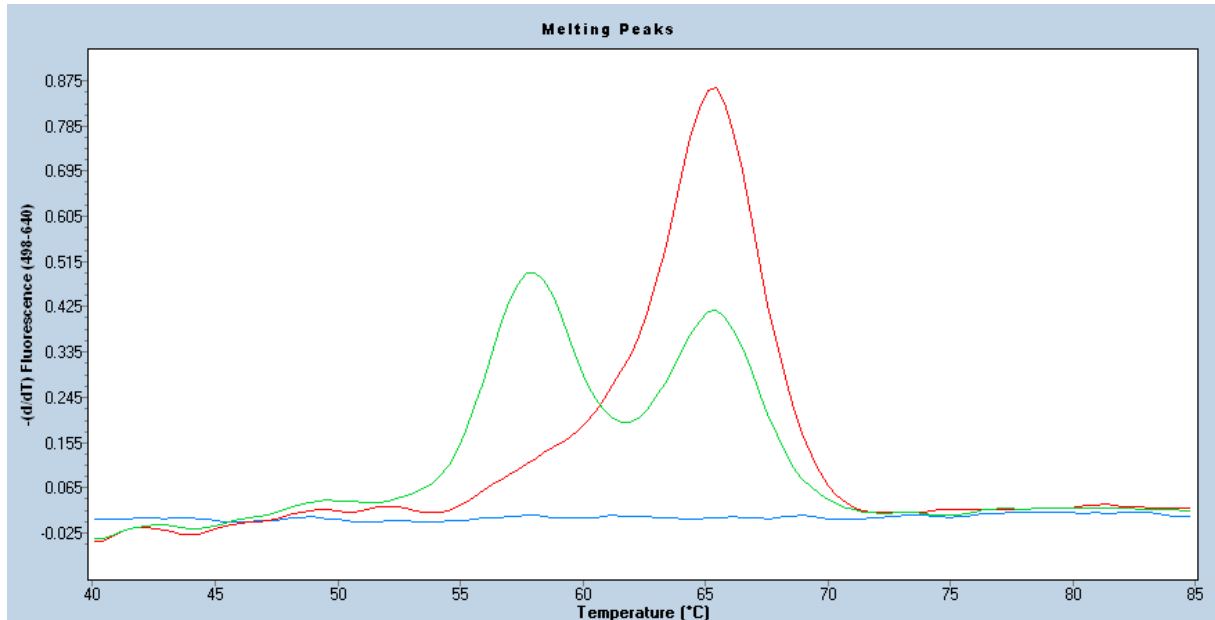


Figure 1 : Melting curve analysis of possible genotypes of the Leiden mutation in the FV gene.

RED : Homozygote **GG wild type** ($T_m(\text{G}) = 65,4 \text{ }^\circ\text{C}$).
GREEN : Heterozygote **GA mutation** = Control contained in the kit ($T_m(\text{A}) = 57,9 \text{ }^\circ\text{C}$).
BLUE : No DNA Control.

Conditions : LC 480 program version 1.5

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 °C.

Restrictions of use

The FV Leiden 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 products is expressly prohibited.

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