

Coagulation Factor XIII (F XIII) Val 34 Leu ToolSet™ for LightCycler™

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

Order#: F XIII 34 - 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-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102)

1. ToolSet contents

Vial	Label	Content	Quantity
			F XIII 34 - 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-DNA Master Hybridization Probes, 10 x conc. Cat.No.: 2 015 102, including 25mM MgCl₂;
LightCycler instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description

This ToolSet is specifically designed for genotyping the Val34Leu (G163T) polymorphism in the Coagulation Factor XIII (F XIII) 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 112 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 F XIII 34 -16, dissolved	2.8
Solvent F XIII 34 -16	9.6
MgCl ₂ 25 mM	1.6 (final 3 mM)
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control F XIII 34 -16	4
Grand Total	20

Use Master Hybridization Probes 10x and MgCl₂ 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102, 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 F XIII 34 - 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 **F XIII Val34Leu** ToolSet™ for LightCycler™ allows detection of the **G163T** single nucleotide polymorphism in the F XIII gene, causing a **Val-to-Leu** exchange in the catalytic A-subunit close to the thrombin activation site which interferes with factor XIII activation. Factor XIIIVal34Leu may thus contribute to the pathogenesis of thrombotic disorders and has been shown to be protective against myocardial infarction and retinal arterial occlusion, while homozygosity for FXIII 34Leu and F XIII Val34Leu / PAI-1 4G5G compound carrier status predispose to early pregnancy loss.

Note : This ToolSet employs the same temperature-time protocol as the PAI-1 4G/5G and MTHFR C677T ToolSets and can be used in the same LightCycler run.

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

4. LightCycler Settings and Experimental Protocol

Denaturation

Cycle Program Data	Value
Cycles	1
Analysis Mode	None
Temperature Targets	Segment 1
Target Temperature (°C)	95
Incubation time (s)	60
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	50		
Analysis Mode	None		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	57	72
Incubation time (s)	1	5	3
Temperature Transition Rate (°/s)	20	20	3
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	80
Incubation time (s)	60	60	0
Temperature Transition Rate (°/s)	20	20	0.1
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)	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 3.3. Use F2/F1 or preferably F2 with colour compensation; with gains F1=1; F2=15. For LC Program Versions 3.5 and higher : use automatic gain control.

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 Val34Leu polymorphism in the F XIII gene. Figure 1 shows a typical result obtained with the F XIII Val34Leu ToolSet™ for LightCycler™ :

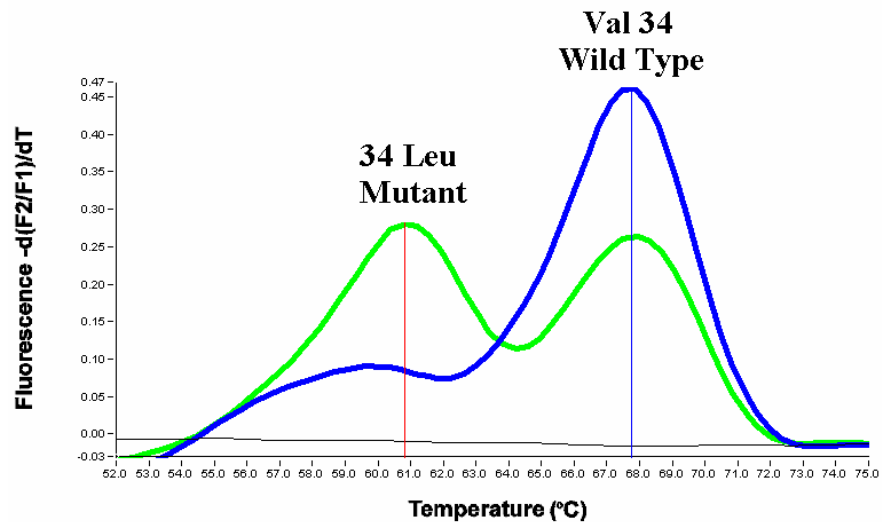


Figure 1 : Melting curve analysis of genotypes of the Val34Leu polymorphism in the F XIII gene.

BLUE : Homozygote for **Val 34 (wild type)** , **BLACK** : No DNA Control.

GREEN : Heterozygote Control contained in the ToolSet, Control **F XIII Val34Leu HET**.

Blue Cursor : $T_m = 67.7$ °C , **Red Cursor** : $T_m = 60.8$ °C.

Conditions : LC program version 3.3 with gain settings F1=1, F2=15, No Color compensation, Digital Filter enabled, Calculation Method : Polynomial, Degrees to average : 8.

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 +/- 0.5 °C. The F XIII Val34Leu 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

ratiogen ToolSets™ for LightCycler™ are produced and marketed under license from Roche Diagnostics GmbH. 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.

The LightCycler technology is licensed from Idaho Technology Inc., Idaho Falls, ID, USA.

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

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