

Human Plasminogen Activator Inhibitor 1 (PAI-1) 4G5G ToolSet™ for LightCycler™

Application on LightCycler 480

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

Order#: PAI-1 4G5G - 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
			PAI-1 4G5G - 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

ToolSet description The ToolSet is specifically designed for genotyping the 4G / 5G insertion/deletion polymorphism in the plasminogen activator inhibitor – 1 (PAI-1) gene promoter 675 bp upstream from the start of transcription by LightCycler PCR with Melting Curve Analysis. The primer pair and fluorescent detection and anchor probes have been optimized for specific amplification of a 134/135 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 PAI-1 4G5G -16, dissolved	2.8
Solvent PAI-1 4G5G -16	8.8
MgCl ₂ 25 mM	2.4
Fast Start DNA Master HybProbes, 10x	2
Total Reaction Mix	16
+ Your DNA or Control PAI-1 4G5G -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.
Use the dissolved heterozygous Control **PAI-1 4G5G** - 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 **PAI-1 4G5G** ToolSet™ for LightCycler™ allows the detection of the **4G / 5G** single nucleotide deletion / insertion in the PAI-1 gene promoter, where the 4G variant is associated with enhanced transcription, enhanced circulating PAI-1 concentrations and a procoagulatory state. Clinically, the **PAI-1 4G5G** polymorphism has been primarily associated with venous and arterial thrombosis and lumen loss after coronary stenting.
Apart from the **coagulation** system, associations with **immune** reactions (prognosis of [meningococcal] sepsis, IgE-mediated allergic diseases and adult periodontitis) and with **obesity** have been observed.

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

4. 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	40		
Analysis Mode	Quantification		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	60	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	40	85
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 of the 4G / 5G polymorphism in the PAI-1 gene promoter. Figure 1 shows a typical result obtained with the PAI-1 4G5G ToolSet™ for LightCycler™ :

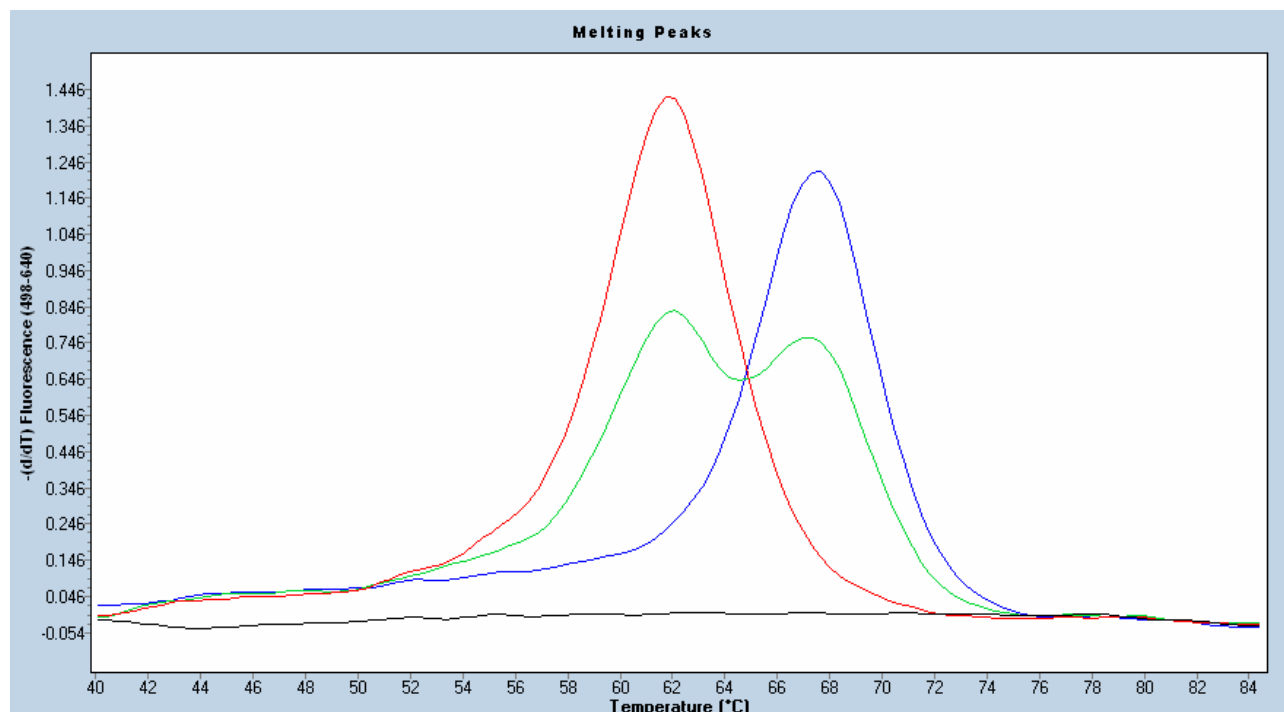


Figure 1 : Melting curve analysis of possible genotypes of the 4G / 5G polymorphism in the PAI-1 gene.

BLUE : Homozygote for **5G** ($T_m = 67.4\text{ }^\circ\text{C}$)

RED : Homozygote for **4G** ($T_m = 62\text{ }^\circ\text{C}$)

GREEN : Heterozygote Control contained in the ToolSet, Control **PAI-1 4G5G**

BLACK : No DNA Control

Conditions : LC 480 program version 1.5, No Color 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 +/- 1.5 °C. The PAI-1 4G5G 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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