

Human Platelet Glycoprotein IA C807T ToolSet™ for LightCycler™

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

Order#: PGPIA 807 - 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
			PGPIA 807 - 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 The ToolSet is specifically designed for genotyping the C to T exchange at nucleotide position 807 of the Human Platelet Glycoprotein IA (Integrin α₂) 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 273 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 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 PGPIA 807 -16 dissolved	2.8
Solvent PGPIA 807 -16	10.4
MgCl ₂ 25 mM	0.8
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control PGPIA 807 -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 **PGPIA 807** - 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 **PGPIA 807** ToolSet™ for LightCycler™ allows the detection of the single point mutation C807T which is associated with variation of platelet alpha 2 beta 1 surface density, correlating with differences in adhesiveness to type-I or type- III collagens. Studies indicate the C807T polymorphism of PGPIa as risk factor for ischemic arterial diseases in younger age groups.

More information is available in the *Genes* section at www.Genes-4U.com

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	58	72
Incubation time (s)	3	15	11
Temperature Transition Rate (°/s)	20	20	2
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)	0	90	0
Temperature Transition Rate (°/s)	20	20	0.2
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)	30
Temperature Transition Rate (°/s)	20
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

Fluorescence display mode

Use preferably F2 with color compensation, or F2/F1 without color compensation.
 Use automatic gain setting with LC program 3.5. Use gains: F1=1; F2=15 for earlier versions of the LC program.

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 position 807 of the Human Platelet Glycoprotein IA (Integrin $\alpha 2$) gene. Figure 1 shows a typical result obtained with the PGPIA 807 ToolSet™ for LightCycler™ :

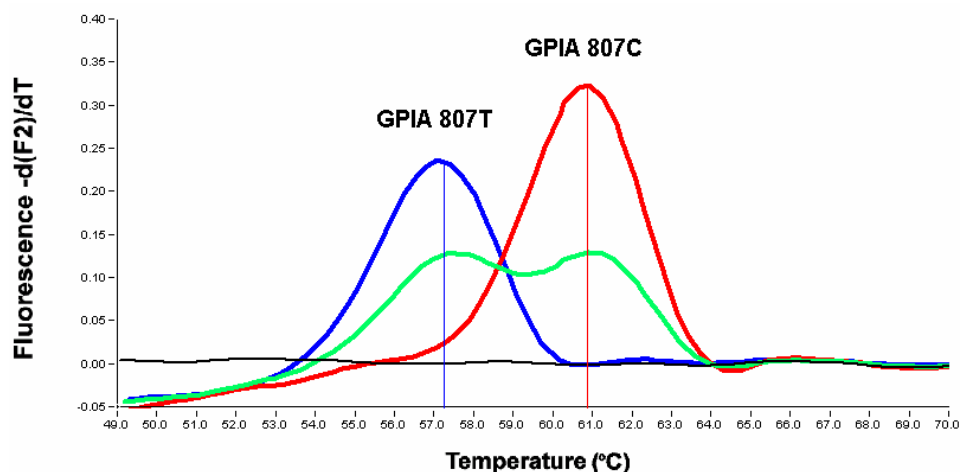


Figure 1 : Melting curve analysis of possible genotypes of PGPIA at nucleotide 807.

BLUE : Homozygote for 807T , **RED** : Homozygous for C807,

GREEN : Heterozygote Control contained in the ToolSet, Control PGPIA **C807T**.

Conditions : LC program version 3.5, Color compensation and Digital Filter enabled,

Calculation Method : Polynomial, Degrees to average : 8

Blue Cursor : $T_m = 57.25$ °C , **Red Cursor** : $T_m = 60.87$ °C.

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 PGPIA 807 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 Genes-4U.

7. Notices to Purchaser

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