

# Platelet Glycoprotein IIIA T393C (HPA-1 a/b = PI<sup>A1</sup>/PI<sup>A2</sup>) ToolSet™ for LightCycler™

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

**Order#: PGPIIIA 393 - 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
			<b>PGPIIIA 393 - 16</b>
<b>1, Red cap</b>	<b>OligoTool</b>	- lyophilized oligos for PCR - contains mutation detection and anchor probe, primers	For 16 tests  Dissolved: 50 µL
<b>2, Green cap</b>	<b>Control</b>	- lyophilized DNA control	Dissolved: 20 µL each
<b>3, Blue cap</b>	<b>LCPCR Solvent</b>	- to dissolve OligoTool <i>only</i>	300 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 PCR grade Water and 25mM MgCl<sub>2</sub>; LightCycler instrument, LightCycler capillaries, DNA extraction materials

## 2. Introduction

### 2.1. Product overview

#### ToolSet description

The ToolSet is specifically designed for genotyping the T to C exchange at nucleotide position 393 of the Human Platelet Glycoprotein IIIA gene encoding the **HPA-1a** (PI<sup>A1</sup>) and **HPA-1b** (PI<sup>A2</sup>) antigenic variants of Human Platelet Glycoprotein IIIA respectively, by LightCycler PCR with Melting Curve Analysis. The primer pair and fluorescent detection and anchor probes are optimized for specific amplification of the segment containing the potentially mutated site and optimal genotype discrimination.

#### Control material

Control DNA, lyophilized : **PGPIIIA T393C Heterozygote**.

#### 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 LCPCR Solvent**. Dissolve the content of the Control tube (Green Cap) with **20 µl of PCR grade Water**.

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 PGPIIIA 393 -16 dissolved *	2.8
<b>PCR grade Water</b>	8
MgCl <sub>2</sub> 25 mM	3.2
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control PGPIIIA 393 -16 **	4
Grand Total	20

Use Master Hybridization Probes 10x , PCR grade Water and MgCl<sub>2</sub> 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102, including 25mM MgCl<sub>2</sub>). For multiple reactions, multiply the indicated volumes appropriately.

**\* Dissolved in LCPCR Solvent    \*\* Dissolved in PCR grade Water**

**Positive Control** Always run a positive control with the samples. Use the dissolved Control **PGPIIIA T393C Het** (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 **PGPIIIA 393** ToolSet™ for LightCycler™ allows detection of the point mutation T393C which encodes antigenic variants of the HPA-1 epitope of glycoprotein IIIa (GPIIIa). The transition from T to C at nucleotide 393 of the GPIIIa gene causes a substitution of leucine to proline at amino acid residue 33 of GPIIIa, converting HPA-1a (PI<sup>A1</sup>) to HPA-1b (PI<sup>A2</sup>). HPA-1a is the alloantigenic determinant implicated in the pathogenesis of most cases of **post-transfusion purpura (PTP)** and **neonatal alloimmune thrombocytopenic purpura (NATP)**.

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	<b>Segment 1</b>
Target Temperature (°C)	95
Incubation time (s)	45
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	<b>50</b>		
Analysis Mode	None		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	55	72
Incubation time (s)	0	6	1
Temperature Transition Rate (°/s)	20	20	<b>3</b>
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	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	40	80
Incubation time (s)	60	180	0
Temperature Transition Rate (°/s)	20	10	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	<b>Segment 1</b>
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

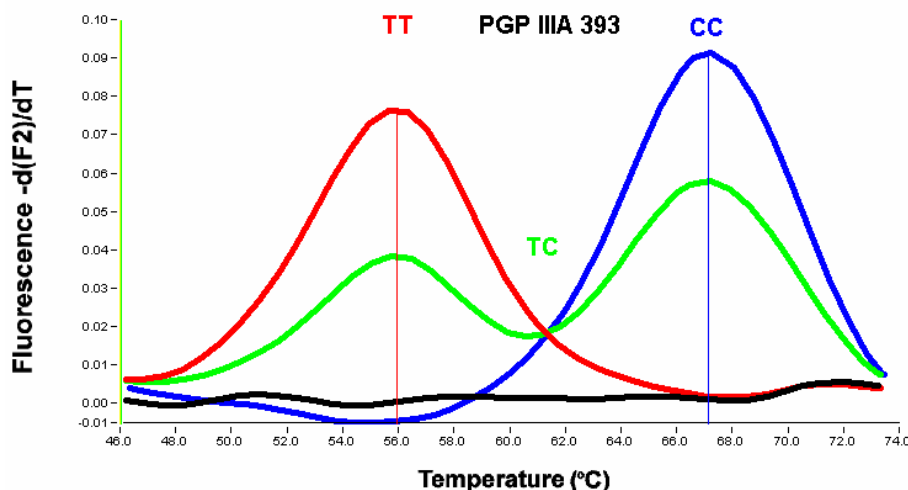
Use preferably F2 with color compensation, or F2/F1 without color compensation.

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 393 of the Human Platelet Glycoprotein IIIA gene. Figure 1 shows a typical result obtained with the PGP IIIA 393 ToolSet™ for LightCycler™ :



**Figure 1 : Melting curve analysis of possible genotypes of PGP IIIA at nucleotide 393.**

**RED** : Homozygote T393. **GREEN** : Heterozygote Control PGP IIIA T393C contained in the ToolSet. **BLUE** : Homozygote for 393C, **BLACK** : No DNA Control.

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

Calculation Method : Polynomial, Degrees to average : 8.

**Red Cursor** :  $T_m = 56\text{ }^{\circ}\text{C}$ , **Blue Cursor** :  $T_m = 67.5\text{ }^{\circ}\text{C}$ .

**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 +/- 1.0 °C. The PGP IIIA 393 / HPA-1 a/b 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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