Protocol uses Fast Start DNA Master SYBR Green !

ratiogen

For life science research purposes only. Not for diagnostic use.
For *IN VITRO* USE ONLY. Version 7, September 2011

HLA B27 ToolSet™ for LightCycler™

Application on LightCycler 480

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: HLA B27

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 SYBR Green, 10 x conc. (Roche Cat.No.: 03003230001) and LightCycler® 480 Multiwell Plates 96, white (Roche Cat.No.: 04729692001)

1. ToolSet contents

Vial	Label	Content	Quantity
			HLA B27
1, Red cap	**OligoTool**	- lyophilized oligos for PCR - contains a primer set for HLA B27 and a primer set for Factor II as amplification control	For 16 tests Dissolved: 50 µL
2 A–B , Green caps	**2 Controls**	- **A** : lyophilized HLA B27 **positive** DNA - **B** : lyophilized HLA B27 **negative** 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 SYBR Green, 10 x conc.Cat.No.: 03003230001, including 25mM $MgCl_2$;
LightCycler® 480 instrument, LightCycler® 480 Multiwell Plates 96 white, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description This ToolSet is specifically designed for detecting presence of the HLA B27 allele of the human HLA B locus by LightCycler PCR with Melting Curve Analysis. Co-amplification of a sequence of the human Factor II gene serves as internal positive control. The two primer pairs have been optimized for specific amplification of HLA B27 (positive if present, yielding a 136 bp segment) and Factor II (yielding a 349 bp segment in absence of HLA B27, but suppressed in presence of HLA B27).

Control material - **A** : HLA B27 **positive** DNA, - **B** : HLA B27 **negative** DNA, both 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.

Protocol uses Fast Start DNA Master SYBR Green !

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 ?
Probes ?

You don't have to add extra primers, they are already contained in the OligoTool.
You don't have to add probes, this setup does not require probes.

Reaction Mix Preparation

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

Reagent	µL
OligoTool HLA B27, dissolved	2.8
Solvent HLA B27	8.8
$MgCl_2$ 25 mM	2.4 (final 4 mM)
Fast Start DNA Master SYBR Green 10x	2
Total Reaction Mix	16
+ Your DNA or Controls HLA B27	4
Grand Total	20

Use Fast Start DNA Master SYBR Green 10x and $MgCl_2$ 25 mM from Roche LightCycler DNA Master SYBR Green, 10 x conc.
(Roche Cat.No.: 03003230001, including 25mM $MgCl_2$).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control

Always run positive controls with the samples. Use the dissolved Controls **A** : **HLA B27 positive DNA** and **B** : **HLA B27 negative DNA** Both contained in the ToolSet (Green Caps).

Negative control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Solvent (Blue Cap).

Note

For optimum performance, centrifuge the filled and sealed microwell plate before inserting it into the LightCycler® 480 instrument.

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 **HLA B27** ToolSet™ for LightCycler™ allows the detection of the **B27 allele** of the HLA-B locus. HLA B27 is strongly associated with Ankylosing Spondylitis (Morbus Bechterew) and related inflammatory conditions.

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

Protocol uses Fast Start DNA Master SYBR Green !

4. LightCycler 480 Settings and Experimental Protocol

For use with LC 480 Program Version 1.5 series. Detection : Dynamic SybrGreen I / HRM 465 – 510 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**
Target Temperature (°C)	**95**	**72**
Incubation time (s) **Hold**	**10**	**15**
Temperature Transition Rate (°/s) **Ramp Rate**	**4.4**	**2.2**
Acquisitions (per °C)	---	---
Secondary Target Temperature (°C)	0	0
Step Size (°C)	0	0
Step Delay (Cycles)	0	0
Acquisition Mode	None	**Single**

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**	**70**	**99**
Incubation time (s) **Hold**	**30**	**30**	---
Temperature Transition Rate (°/s) **Ramp Rate**	**4.4**	**2.2**	**0.1**
Acquisitions (per °C)	---	---	**6**
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

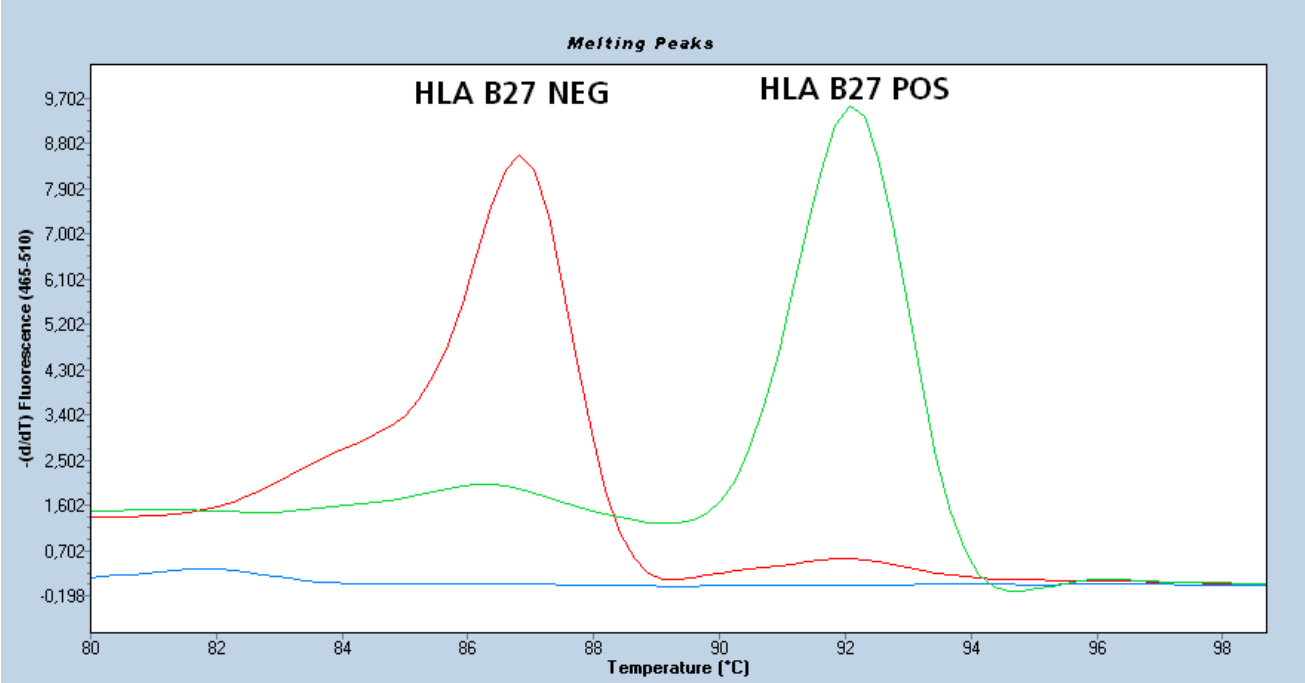
Cycle Program Data	**Value**
Cycles	**1**
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature (°C)	**40**
Incubation time (s) **Hold**	**30**
Temperature Transition Rate (°/s) **Ramp Rate**	**2.2**
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

Protocol uses Fast Start DNA Master SYBR Green !

5. Typical results

Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples. The melting peaks allow discrimination between presence or absence of the HLA B27 allele with the Factor II amplicon peak serving as positive control. The Figure shows a typical result obtained with the HLA B27 ToolSet™ for LightCycler™ :



Melting curve analysis of HLA B27 and Factor II amplicons.

GREEN : HLA B27 Positive Control , Tm = 92.1 °C, with Factor II amplicon absent / minimal.

RED: HLA B27 Negative Control, Tm = 86.7 °C, with Factor II amplicon present

BLUE : No DNA Control.

Note : In presence of HLA B27, amplification of Factor II is markedly suppressed.

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 HLA B27 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's 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 expressively 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 expressively prohibited.

How to contact ratiogen

E-mail info@ratiogen.com

Internet http://www.ratiogen.com