

HLA B5701 ToolSet™ for LightCycler™

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: HLA B5701 - 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 SYBR Green, 10 x conc.** (Roche Cat.No.: 03003230001)

1. ToolSet contents

Vial	Label	Content	Quantity
			HLA B27
1, Red cap	OligoTool	- lyophilized oligos for PCR - contains a primer set for HLA B5701 and a primer set for the amplification control	For 16 tests Dissolved: 50 µL
2 A–B , Green caps	2 Controls	- A : lyophilized HLA B5701 positive DNA - B : lyophilized HLA B5701 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 MgCl₂;
LightCycler instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description This ToolSet is specifically designed for detecting presence of the HLA B5701 allele of the human HLA B locus (B570101, B570102 and B570103) by LightCycler PCR with Melting Curve Analysis. Co-amplification of another gene sequence serves as internal positive control. Primers have been optimized for specific amplification of HLA B5701 (yielding a 130 bp segment in presence of HLA B5701) and a control gene (yielding a 349 bp segment in absence of HLA B5701, but suppressed in presence of HLA B5701).

Control material - **A** : HLA B5701 **positive** DNA, - **B** : HLA B5701 **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.

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 briefly.
2. Add Solvent into OligoTool tube and Control tube as above.
3. Recap tubes, vortex gently.
4. Before opening tubes, centrifuge them briefly.
5. Proceed to PCR setup.

PCR Setup

1. Make sure the LightCycler is programmed correctly (see next page).
2. Place the required number of capillaries in the LightCycler rotor.
3. Add Your DNA or Controls (**4 µL**) into the LightCycler capillaries.
4. Prepare the Reaction Mix (see below). Keep in a cooled metal block (+2 to +8 °C).
5. Add **16 µL** of Reaction Mix to each capillary and stopper the capillaries.
6. Centrifuge the LightCycler rotor and place it in the LightCycler.
7. Start the LightCycler PCR.

Note : Strictly adhere to this order of steps and make no stops in between.

Reaction Mix Preparation For **1 (One)** reaction, prepare the Reaction Mix as shown in the following table :
For **N** reactions, **multiply** the indicated volumes **by (N+1)**.

Reagent	µL
OligoTool HLA B5701, dissolved	2.8
Solvent HLA B5701	8.8
MgCl ₂ 25 mM	2.4 (final 4 mM)
Fast Start DNA Master SYBR Green 10x	2
Total Reaction Mix	16

Use Fast Start DNA Master SYBR Green 10x and MgCl₂ 25 mM from Roche LightCycler DNA Master SYBR Green, 10 x conc.
(Roche Cat.No.: 03003230001, including 25mM MgCl₂).

Positive Control Always run positive controls with the samples. Use the dissolved Controls **A : HLA B5701 positive DNA** and **B : HLA B5701 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).

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 B5701 ToolSet™** for LightCycler™ allows the detection of **B5701 alleles (B570101, B570102 and B570103)** of the HLA-B locus which are strongly associated with Hypersensitivity to the antiretroviral drug **Abacavir®**.

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

4. LightCycler Settings and Experimental Protocol

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)	600
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	40		
Analysis Mode	None		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	70	72
Incubation time (s)	10	10	20
Temperature Transition Rate (°/s)	20	20	20
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	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)	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

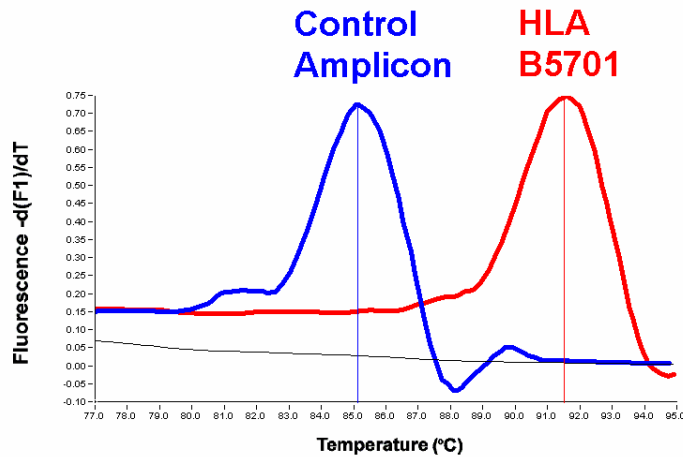
Developed with LC Program Version 3.5.

For readout use channel F1(Fluorescein).

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 B5701 allele with the Control amplicon peak serving as positive control. The Figure shows a typical result obtained with the HLA B5701 ToolSet™ for LightCycler™ :



Melting curve analysis of HLA B5701 and Control amplicons.

BLUE : HLA B5701 **negative** Control with Control amplicon present
RED : HLA B5701 **positive** Control with Control amplicon absent
BLACK : No DNA Control

Blue Cursor (Control amplicon): $T_m = 85.1\text{ }^{\circ}\text{C}$
Red Cursor (HLA B5701): $T_m = 91.5\text{ }^{\circ}\text{C}$.

Note : In presence of HLA B5701, amplification of the Control gene is suppressed.

Conditions : LC program version 3.5 with automatic gain setting, 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 +/- 1.0 °C. The HLA B5701 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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