

HLA B51 ToolSet™ for LightCycler™ (Morbus Behçet)

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: HLA B51

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 B51
1, Red cap	OligoTool	- lyophilized oligos for PCR - contains a primer set for HLA B51 and a primer set for HLA-H as amplification control	For 16 tests Dissolved: 50 µL
2 A-B , Green caps	2 Controls	- A : lyophilized HLA B51 positive DNA - B : lyophilized HLA B51 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 B51 allele of the human HLA B locus by LightCycler PCR with Melting Curve Analysis. Co-amplification of a sequence of the human HLA-H gene serves as internal positive control. The two primer pairs have been optimized for specific amplification of HLA B51 (positive if present, yielding a 205 bp segment) and HLA-H (yielding a 390 bp segment in absence of HLA B51, but suppressed in presence of HLA B51).

Control material

- **A** : HLA B51 **positive** DNA, - **B** : HLA B51 **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 24 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 extra primers, they are already contained in the OligoTool.
Probes ? 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 B51, dissolved	2.8
Solvent HLA B51	10.4
MgCl ₂ 25 mM	0.8 (final 2 mM)
Fast Start DNA Master SYBR Green 10x	2
Total Reaction Mix	16
+ Your DNA or Controls HLA B51	4
Grand Total	20

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).
 For multiple reactions, multiply the indicated volumes appropriately.

Positive Control Always run positive controls with the samples. Use the dissolved Controls **A : HLA B51 positive DNA** and **B : HLA B51 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 B51 ToolSet™** for LightCycler™ allows the detection of the **B51 allele** of the HLA-B locus. HLA B51 is strongly associated with e.g. **Aphthosis, Uveitis and Iritis (Morbus Behçet)** and related inflammatory conditions.

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	
Target Temperature (°C)	95	71	
Incubation time (s)	10	30	
Temperature Transition Rate (°/s)	20	20	
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)	30	30	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).

