

# CYP21B fl/del ToolSet™ for LightCycler™ (Detection of full length/deletion copies of the functional CYP21B gene)

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

**Order#: CYP21B fl/del**

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>CYP21B fl/del</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 A-B, Green cap</b>	<b>Control</b>	- <b>A</b> : CYP21B full length <b>positive</b> DNA, lyophilized - <b>B</b> : CYP21B full length <b>negative</b> DNA, lyophilized	Dissolved: 20 µL each
<b>3, Blue cap</b>	<b>Solvent</b>	- 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 MgCl<sub>2</sub>;  
LightCycler instrument, LightCycler capillaries, DNA extraction materials

## 2. Introduction

### 2.1. Product overview

**ToolSet description** This ToolSet is designed for genotyping the human **CYP21B** gene (**21-Hydroxylase**) for presence of at least one full length (fl) allele versus homozygous large deletions or isolated 8 bp deletions by LightCycler PCR with Melting Curve Analysis. The primer pair co-amplifies a 215 bp segment of the functional gene CYP21B and a 207 bp segment of the pseudogene CYP21A. Intact CYP21B amplicons, if present, are monitored by a LC Red 640 labelled fluorescent probe complementary to the functional gene. As an internal positive control, always present pseudogene amplicons are monitored by a LC Red 705 labelled fluorescent probe complementary to the 8 bp deletion.

**Control material** **A** : CYP21B fl **positive** DNA, - **B** : CYP21B fl **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 ml of Solvent**.  
**Dissolve** the content of the **Control** tubes (Green Cap) with **20 ml 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 CYP21B fl/del, dissolved	2.8
Solvent CYP21B fl/del	10.4
MgCl <sub>2</sub> 25 mM	0.8 (final 2 mM)
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Controls CYP21B fl/del	4
Grand Total	20

Use Master Hybridization Probes 10x 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.

**Positive Control** Always run the positive controls with the samples. Use the dissolved Controls **A** : CYP21B fl **positive** DNA, - **B** : CYP21B fl **negative** DNA (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 **CYP21B fl/del ToolSet™ for LightCycler™** has been specifically designed for the detection of full length versus large / 8bp deletion copies of the functional CYP21B gene that entail deletions of the CYP21B region homologous to the region where the pseudogene CYP21A carries an 8bp deletion. Note that by its nature the assay cannot distinguish between carriership of one or two ("wild type") full length copies of the CYP21B gene.

**About 30 % of patients with CYP21 deficiency / Adrenogenital Syndrome** carry large or 8 bp deletions as detected by the CYP21 fl/del ToolSet™ for LightCycler™.

DNA samples that contain at least one copy of the full-length CYP21B gene as evidenced by the CYP21B fl/del ToolSet™ for LightCycler™ should be subjected to **further analysis using the CYP21 B ToolSet designed for point mutations in CYP21B**.

More information is available in the *Genes* section at [www.Genes-4U.com](http://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	<b>Segment 1</b>
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	<b>45</b>		
Analysis Mode	None		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	60	72
Incubation time (s)	2	<b>10</b>	<b>10</b>
Temperature Transition Rate (°/s)	20	<b>20</b>	<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	85
Incubation time (s)	30	60	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	<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

### LC Program Version and Fluorescence Display Mode

Developed with LC Program Version 3.5.

Use F2/F1 and F3/F2.

## 5. Typical results

### Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples. Melting peaks allow discrimination of Full Length and Deletion genotypes of the human CYP21B (21-Hydroxylase) gene with the CYP21A (pseudogene) serving as positive control. Figure 1 shows a typical result obtained with the CYP21B fl/del ToolSet™ for LightCycler™ :

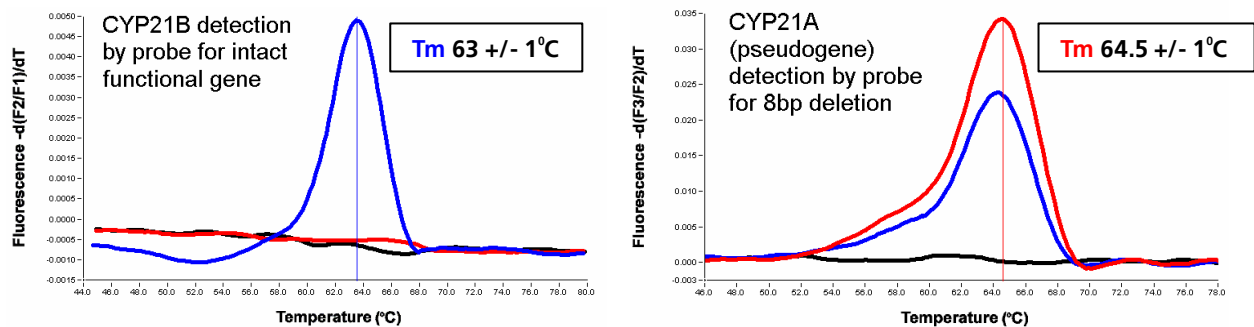


Figure 1 : Melting curve analysis of full length / deletion (fl/del ) genotypes of the CYP21B gene.

#### Left : Channel F2/F1 (functional gene probe)

**BLUE** : DNA with at least one functional CYP21B gene present = Control **A** : CYP21B fl **positive** DNA  
**RED** : DNA homozygote for large deletion in the CYP21B gene = Control **B** : CYP21B fl **negative** DNA  
**BLACK** : No DNA Control.

#### Right : Channel F3/F2 (8bp deletion probe)

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation, Digital Filter enabled, Calculation Method : Polynomial, Degrees to average : 10.

**Note** : The values for the respective melting temperatures may vary for +/- 2.5 °C between different experiments. The CYP21B fl/del ToolSet™ has been developed for and validated with the LightCycler™ and its original accessory materials and reagents. Genes-4U has not validated the ToolSet with other instruments, accessories or reagents.

### Interpretation and suggested further analyses :

**Melting peak in channels F2/F1 and F3/F2 :** **No evidence for homozygote large deletion or 8bp deletion.** Proceed with analysis of further CYP21B mutations with the ToolSets CYP21B I2G, I172N, V281L, Q318X, R356W, P30L, E6 cluster, F306+T and P453S.

**Melting peak only in channel F3/F2 :** **Evidence for a homozygote large deletion or 8bp deletion.** Proceed with the CYP21B del 8bp ToolSet to differentiate 8bp deletion from large deletion.

**No melting peak in channel F3/F2:** PCR not successful. Repeat with fresh DNA / fresh reagents.

## 7. Notices to Purchaser

### Licenses and Trademarks, Prohibition of Resale

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