

# Leukotriene C4 Synthase (LTC4S) A-444C ToolSet™ for LightCycler™

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

**Order#: LTC4S -444 - 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>LTC4S -444 - 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 heterozygous DNA	Dissolved: 20 µL
<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 specifically designed for genotyping the A-444C promoter polymorphism in the Leukotriene C4 Synthase (LTC4S) gene by LightCycler PCR with Melting Curve Analysis. The primer pair and fluorescent detection and anchor probes have been optimized for specific amplification of a 286 bp segment containing the potentially mutated site and for optimal genotype discrimination.

#### Control material

Heterozygous control DNA, 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 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 LTC4S -444 -16, dissolved	2.8
Solvent LTC4S -444 -16	9.6
MgCl <sub>2</sub> 25 mM	1.6 (final 3 mM)
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control LTC4S -444 -16	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 a positive control with the samples. Use the dissolved heterozygous Control LTC4S -444 -16 DNA (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 **LTC4S A-444C** ToolSet™ for LightCycler™ allows detection of the **A-444C** single nucleotide polymorphism in the LTC4S gene promoter. The C allele causes enhanced transcription, particularly in presence of glucocorticoids, and thus a propensity for enhanced generation of LTC4 and other sulfidoleukotrienes. Clinically, the **LTC4S -444C** allele has been associated with Aspirin-induced asthma and with stronger effects of LTC4-receptor-antagonists like Pranlukast and Zafirlukast.

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)	120
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>60</b>		
Analysis Mode	None		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	<b>57</b>	72
Incubation time (s)	1	<b>10</b>	<b>13</b>
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	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	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	<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

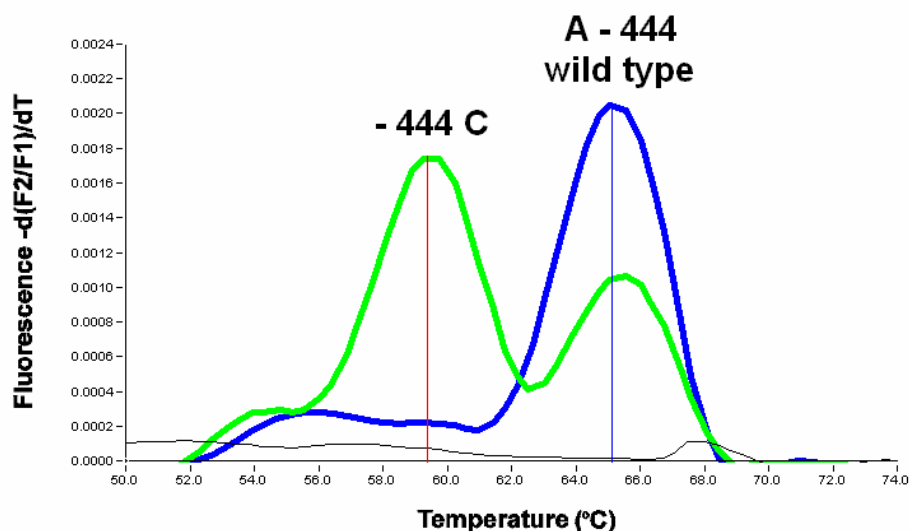
### LC Program Version and Fluorescence Display Mode

Developed with LC Program Version 3.3. Use F2/F1 or preferably F2 with colour compensation; with gains F1=1; F2=15. For LC Program Versions 3.5 and higher : use automatic gain control.

## 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 of the A-444C polymorphism in the LTC4S gene. Figure 1 shows a typical result obtained with the LTC4S A-444C ToolSet™ for LightCycler™ :



**Figure 1 : Melting curve analysis of possible genotypes of the A-444C polymorphism in the LTC4S gene.**

**BLUE** : Homozygote for **A-444 (wild type)** , **BLACK** : No DNA Control.

**GREEN** : Heterozygote Control contained in the ToolSet, Control **LTC4S A-444C HET**.

**Blue Cursor** :  $T_m = 65.1\text{ }^{\circ}\text{C}$  , **Red Cursor** :  $T_m = 59.4\text{ }^{\circ}\text{C}$ .

Conditions : LC program version 3.5, no Color compensation but Digital Filter enabled, Degrees to average : 10.

**Note ! Calculation Method : Polynomial with Background correction : Set Lower background cursors to 40 / 75.0 °C, Upper background cursors to 65.0 / 80 °C. Other calculation modes may display suboptimally.**

**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 +/- 0.5 °C. The LTC4S A-444C 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 Genes-4U.

## 7. Notices to Purchaser

### Licenses and Trademarks, Prohibition of Resale

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