

# TLR4 Asp 299 Gly ToolSet™ for LightCycler™

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

**Order#: TLR4 299 - 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>TLR4 299 - 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** The ToolSet is specifically adapted for genotyping TLR4 Amino Acid position 299 by LightCycler PCR with Melting Curve Analysis. Fluorescent detection and anchor probes and the primer pair have been optimized for specific amplification of targets and 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 TLR4 299-16 dissolved	2.8
Solvent TLR4 299-16	9.2
MgCl <sub>2</sub> 25 mM	2.0 (final 3.5 mM)
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control TLR4 299-16	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 TLR4 299-16 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 TLR4 Asp 299 Gly ToolSet™ for LightCycler™ allows the detection of the single point mutation resulting in the Asp 299 Gly variant of the TLR4 gene which is associated with diminished responsiveness to bacterial LPS.

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

**Note** : This ToolSet uses the same Time-Temperature-Protocol as the TLR4 Thr 399 Ile ToolSet and can be used in the same LC run.

## 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)	20.0
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

### Amplification

Cycle Program Data	Value		
Cycles	50		
Analysis Mode	None		
Temperature Targets	<b>Segment 1</b>	<b>Segment 2</b>	<b>Segment 3</b>
Target Temperature (°C)	95	50	72
Incubation time (s)	5	10	30
Temperature Transition Rate (°/s)	20.0	20.0	20.0
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)	60	120	0
Temperature Transition Rate (°/s)	20.0	20.0	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)	20.0
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

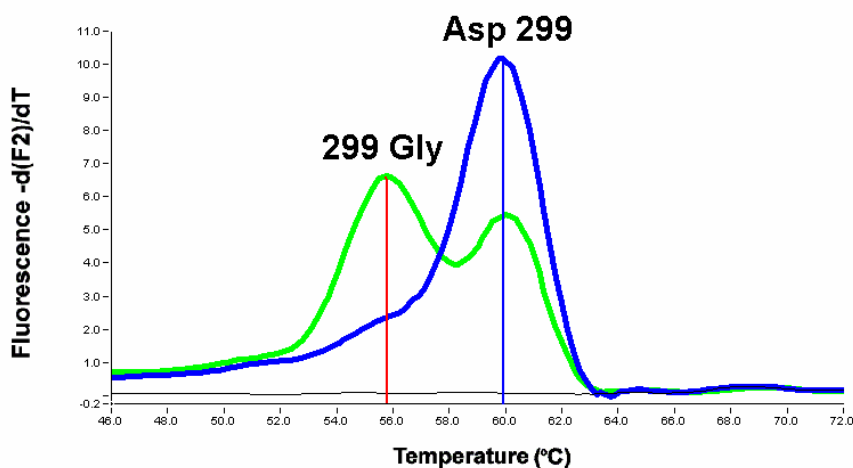
### Fluorescence display mode

With LC program 3.3 use F2/F1 or preferably F2 with colour compensation; gains: F1=1; F2=15.  
With LC program 3.5 use automatic gain control, preferably with colour compensation.

## 5. Typical results

### Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples. The melting peaks allow discrimination between the homozygous (wild type or mutant) and the heterozygous samples. Figure 1 shows a typical result obtained with the TLR4 Asp 299 Gly ToolSet™ for LightCycler™ :



**Figure 1 : Melting curve analysis of possible genotypes of the TLR4 sequence at AA 299.**

**BLUE** : Homozygote for Asp 299 (Wild Type), **BLACK** : No DNA Control  
**GREEN** : The Heterozygote Asp 299 Gly Control contained in the ToolSet, Control TLR4 299-16.  
Conditions : Color compensation and Digital Filter enabled,  
Calculation Method : Polynomial, Degrees to Average : 8.0  
Red Cursor :  $T_m = 55.8\text{ }^{\circ}\text{C}$ , Blue Cursor :  $T_m = 59.9\text{ }^{\circ}\text{C}$

**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 TLR4 Asp 299 Gly 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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### How to contact Genes-4U

E-mail [info@Genes-4U.com](mailto:info@Genes-4U.com)

Internet <http://www.Genes-4u.com>