IL-10 C-819T ToolSet™ for LightCycler™

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

Order#: IL10 -819 - 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
			IL10 -819 - 16
1, Red cap	**OligoTool**	- lyophilized oligos for PCR - contains mutation detection and anchor probe, primers	For 16 tests Dissolved: 50 µL
2, Green cap	**Control**	- lyophilized heterozygous 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 Hybridization Probes, 10 x conc.Cat.No.: 2 015 102, including 25mM $MgCl_2$; LightCycler instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description

The ToolSet is specifically adapted for genotyping the IL - 10 promoter nucleotide position C-819T 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 times.

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 ?
Probes ?

You don't have to add primers.
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 IL-10 C–819T dissolved	2.8
Solvent IL-10 C–819T	10.4
$MgCl_2$ 25 mM	0.8
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control IL-10 C–819T	4
Grand Total	20

Use Master Hybridization Probes 10x and $MgCl_2$ 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102, including 25mM $MgCl_2$).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control

Always run a positive control with the samples.
Use the dissolved heterozygous Control IL-10 C–819T 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 IL-10 C–819T ToolSet™ for LightCycler™ allows the detection of the single point mutation at promoter position –819 which has been associated with altered transcription of the Interleukin-10 gene, altered generation of the gene product – i.e. the cytokine interleukin-10 - and with a variety of disease states.

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

Note : This ToolSet uses the same temperature-time protocol as IL-10 G-1082A.

4. LightCycler Settings and Experimental Protocol

Denaturation

Cycle Program Data	**Value**
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
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	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	50	72
Incubation time (s)	1	10	10
Temperature Transition Rate (°/s)	20.0	20.0	5.0
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	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	35	80
Incubation time (s)	60	120	0
Temperature Transition Rate (°/s)	20.0	20.0	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	**Segment 1**
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

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 homozygous (wild type or mutant) and the heterozygous samples. Figure 1 shows a typical result obtained with the **IL-10 C–819T** ToolSet™ for LightCycler™ :

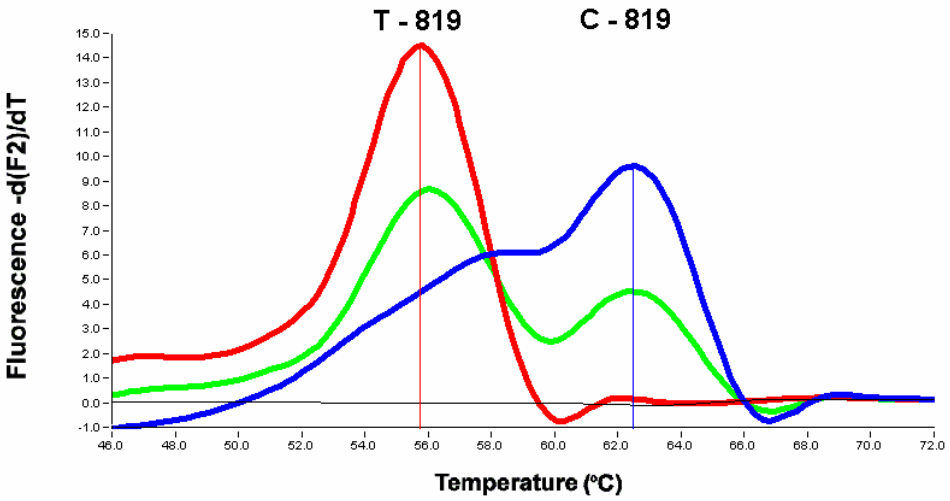

Figure 1 : Melting curve analysis of the three possible genotypes of the IL-10 sequence at nt –819.

BLUE : Homozygote for C –819 (**wild type**), **RED** : Homozygous for – 819 T ,
GREEN : The C–819T Heterozygote Control contained in the ToolSet, Control IL-10 C–819T -16.
Conditions : LC Program 3.3, Color compensation and Digital Filter enabled,
Calculation Method : Polynomial, Degrees to Average : 11.
Red Cursor : T_m = 55.7 ^{0}C, Blue Cursor : T_m = 62.5 ^{0}C

Note : The values for the respective melting temperatures may vary for +/- 2.5 ^{0}C between different experiments. The Delta T between the melting peaks for different genotypes may vary +/- 1.0 ^{0}C. The IL-10 C–819T 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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