ratiogen

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For *IN VITRO* USE ONLY. Version 1, February 2008

Interferon γ A874T ToolSet™ for LightCycler™

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

Order#: IFNG 874 - 96

1 ToolSet for 96 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
			IFNG 874 - 96
1, Red cap	**OligoTool**	- lyophilized oligos for PCR - contains primers	For 96 tests Dissolved: 300 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 SYBR Green, 10 x conc., Cat.No.: 03003230001, including 25mM $MgCl_2$; LightCycler instrument & capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description

This ToolSet is specifically designed for genotyping the A874T polymorphism in the Interferon γ gene flanking a polymorphic CA repeat in intron 1 by multiplex allele-specific LightCycler PCR with Melting Curve Analysis.
Primers are optimized for the specific amplification of 82 bp (T allele) and 108 bp segments (A allele) allowing 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 **300 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 IFNG 874-96, dissolved	2.8
Solvent IFNG 874-96	3.8
$MgCl_2$ 25 mM	0.4 (final **2** mM)
Fast Start DNA Master SYBR Green 10x	1
Total Reaction Mix	**8**
+ Your DNA or Control IFNG 874-96	**2**
Grand Total	10

Note :

This test uses a total volume of only 10 µL per capillary !

Use Fast Start DNA Master SYBR Green 10x and $MgCl_2$ 25 mM from Roche LightCycler DNA Master SYBR Green, 10 x conc.
(Roche Cat.No.: 03003230001, 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 IFNG A874T heterozygous Control 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 **IFNG A874T** ToolSet™ for LightCycler™ allows detection of the **A→T** single nucleotide polymorphism at position 874 in intron 1 of the Interferon γ gene. This polymorphic site may affect IFN γ gene expression as it coincides with a putative NFκB binding site.

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

4. LightCycler Settings and Experimental Protocol

Denaturation and FastStart Enzyme Activation

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	**45 DO NOT EXCEED !**		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**60**	72
Incubation time (s)	10	**5**	5
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	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	**40**	**99**
Incubation time (s)	30	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	**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 and automatic gain control.
For readout use channel F1(Fluorescein).

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 **A874T** polymorphism in the **IFNγ** gene. Figure 1 shows a typical result obtained with the **IFNG A874T** ToolSet™ for LightCycler™ :

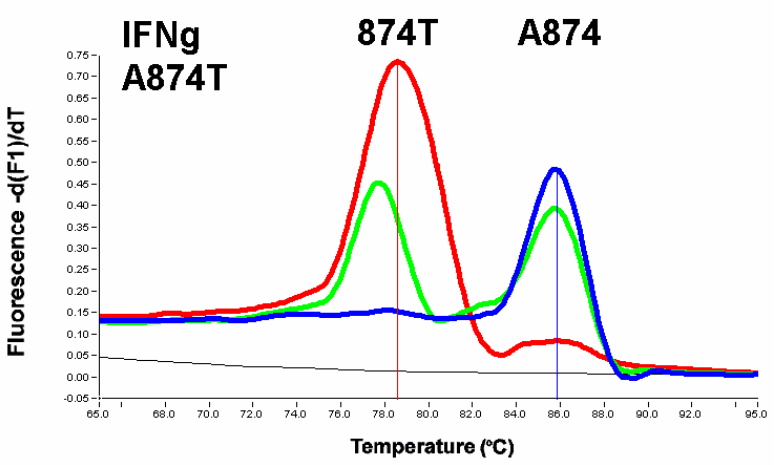

Figure 1 : Melting curve analysis of possible genotypes at position 874 of the human IFNγ gene

BLUE : Homozygote **AA 874** DNA (wild type)
GREEN : Heterozygote **A 874** T Heterozygote Control DNA contained in the ToolSet,
RED : Homozygote **874 TT** DNA
BLACK : No DNA Control.

Blue Cursor : T_m = 85.8 °C ; **Red Cursor** : T_m = 78.6 °C

Note : Heterozygotes show slightly Tm difference between peaks than Homozygotes

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation, Digital Filter enabled, Degrees to average : 7.0. Calculation Method : Polynomial.

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 IFNG A874T 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

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How to contact ratiogen

E-mail info@ratiogen.com

Internet http://www.ratiogen.com