ratiogen

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For *IN VITRO* USE ONLY. Version 1, May 2012

DPD exon 14 skipping ToolSet™ for LightCycler™

(Dihydropyrimidine Dehydrogenase (DPD), 5-fluorouracil (5-FU))

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

Order#: DPD e14 - 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
			DPD e14 - 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 heterozygote 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 This ToolSet is specifically designed for genotyping the IVS14+1 G→A mutation in the DPD gene causing exon 14 skipping by LightCycler PCR with Melting Curve Analysis. The primer pair and fluorescent detection and anchor probes have been optimized for specific amplification of a 199 bp segment containing the potentially mutated site 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 1 week, or at -20°C for longer periods (months), protected from light. Avoid freezing and thawing more than 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 ?
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 DPD e14 -16, dissolved	2.8
Solvent DPD e14 -16	10.0
$MgCl_2$ 25 mM	1.2 (final **2.5** mM)
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control DPD e14 skip HET –16	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 DPD e14 skip 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 **DPD Exon 14 skipping** ToolSet™ for LightCycler™ allows detection of the **G→A** mutation at **IVS14+1** in the DPD gene causing a **loss of DPD activity**. Although several activity impairing mutations have been described in the DPD gene, the exon 14 skipping mutation is the most common mutation and is found in 40-50% of patients experiencing toxicity on 5-FU treatment.

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	**Segment 1**
Target Temperature (°C)	95
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

Amplification **Note : Due to the low Tm of fluorescent probes, no amplification signal is visible**

Cycle Program Data	Value		
Cycles	**45**		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**58**	72
Incubation time (s)	5	**5**	10
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	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**40**	**80**
Incubation time (s)	30	120	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 fluorescence display use F2/F1, or F2 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 possible genotypes at the **exon 14 skipping** mutation site in the **DPD** gene. Figure 1 shows a typical result obtained with the **DPD exon 14 skipping** ToolSet™ for LightCycler™ :

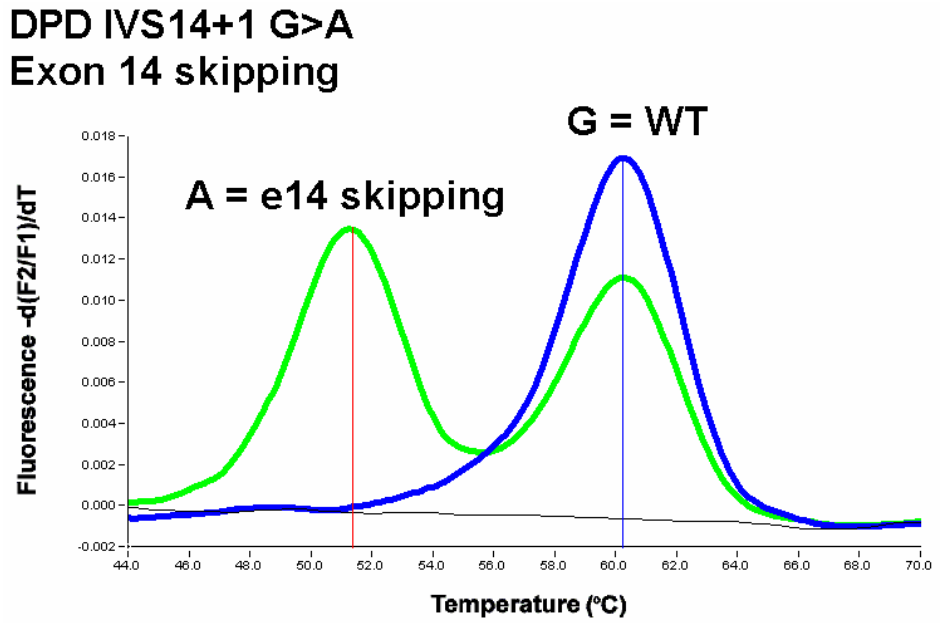

Figure 1 : Melting curve analysis of genotypes at IVS14+1 G→A of the human DPD gene

BLUE : **Homozygote GG DNA** (wild type)
GREEN : **Heterozygote GA DNA** (Heterozygote Control DNA contained in the ToolSet)
BLACK : No DNA Control.

Blue Cursor : T_m = 60.2 °C ; **Red Cursor** : T_m = 51.4 °C

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation, Digital Filter enabled, Degrees to average : 8.0. 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 +/- 1.0 °C. The DPD Exon 14 skipping 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

How to contact ratiogen

E-mail info@ratiogen.com

Internet http://www.ratiogen.com