ratiogen

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For *IN VITRO* USE ONLY. Version 5, November 2024

UGT1A6 T181A (nt A541G) ToolSet™ for LightCycler™

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: UGT1A6 181 - 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
			UGT1A6 181 - 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 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 amino acid T181A respectively nucleotide A541G polymorphism in the UGT1A6 gene 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 ?
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 UGT1A6 181–16, dissolved	2.8
Solvent UGT1A6 181-16	8.8
$MgCl_2$ 25 mM	2.4
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control UGT1A6 181-16, dissolved	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 UGT1A6 181-16 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 UGT1A6 T181A ToolSet™ for LightCycler™ allows the detection of the A541G nucleotide exchange = T181A amino acid exchange in the UGT1A6 gene which is usually linked with the A552C nucleotide exchange = R184S amino acid exchange and associated with diminished activity of the UGT1A6 gene product – i.e. the glucuronidation enzyme UGT1A6. UGT1A6 metabolizes mainly planar phenols such as 4-naphtol, 4-methylphenol and planar arylamines like 1- and 2-naphtylamine. Medical drugs such as aspirin and other NSAID's are also substrates for UGT1A6, and the protective effect of aspirin on colon adenoma risk was recently shown to be restricted to carriers of UGT1A6 181A mutant alleles (Cancer Res. 2001 61(9):3566-9; PubMedID : 11325819).

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)	120
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	45		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	53	72
Incubation time (s)	1	12	18
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	40	85
Incubation time (s)	60	60	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	**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

Fluorescence display mode

Preferably use LC Program Version 3.3 with F2/F1 or F2 with colour compensation and 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 UGT1A6 T181A ToolSet™ for LightCycler™ :

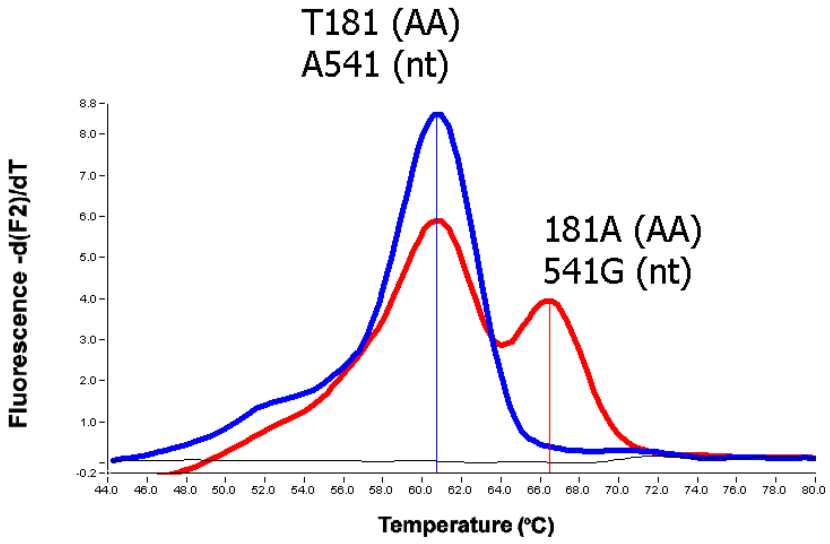

Figure 1 : Melting curve analysis of two possible genotypes of the UGT1A6 sequence.

BLUE: Homozygote wild type T181 DNA.
RED : The Heterozygote T181A Control DNA contained in the ToolSet (Green Cap tube).
Black : NO DNA Control.

Conditions : LC Program 3.3, Color compensation and Digital Filter enabled, Calculation Method : Polynomial, Degrees to Average : 9. Red Cursor : T_m = 66.4 °C, Blue Cursor : T_m = 60.7 °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 +/- 1.0 °C. The UGT1A6 T181A 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.

6. Notices to Purchaser : Licenses and Trademarks, Prohibition of Resale

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