ratiogen

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For *IN VITRO* USE ONLY. Version 1, August 2018

APOB100 R3500Q ToolSet™ for LightCycler™

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

Order#: APOB - 3500 - 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 FastStart DNA Master HybProbe**, 10 x conc. (Roche Cat.No.: 03003248001)

1. ToolSet contents

Vial	Label	Content	Quantity
			APOB - 3500 - 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 wild type 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 FastStart DNA Master Hybridization Probes, 10 x conc.Cat.No.: 03003248001, 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 R3500Q variant of APOB 100 (rs5742904) by LightCycler PCR with Melting Curve Analysis. The primer pair (yielding a 305 bp amplicon) and fluorescent detection and anchor probes are optimized for specific amplification of targets and optimal genotype discrimination.

Control material Wild type 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 24 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 APOB-3500 dissolved	2.8
Solvent APOB-3500	9.6
$MgCl_2$ 25 mM	1.6 (final 3mM)
FastStart DNA Master HybProbe, 10x	2
Total Reaction Mix	16
+ Your DNA or APOB-3500	4
Grand Total	20

Use FastStart DNA Master HybProbe 10x and $MgCl_2$ 25 mM from Roche LightCycler FastStart DNA Master HybProbe, 10 x conc. (Roche Cat.No.: 03003248001, 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 wild type Control APOB-3500 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 APOB100-3500 ToolSet™ for LightCycler™ allows the detection of the R3500Q variant of APOB100 which is associated with decreased (if R3500Q) respectively strongly decreased (if 3500QQ) LDL affinity of the APOB100 protein.

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

4. LightCycler Settings and Experimental Protocol

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

Amplification

Cycle Program Data	**Value**		
Cycles	55		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	60	72
Incubation time (s)	5	10	15
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	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	40	85
Incubation time (s)	30	60	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

Fluorescence display mode

Use F2/F1 or preferably F2 with colour compensation. For LC Program Versions 3.3 or lower : 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 APOB100 R3500Q ToolSet™ for LightCycler™ :

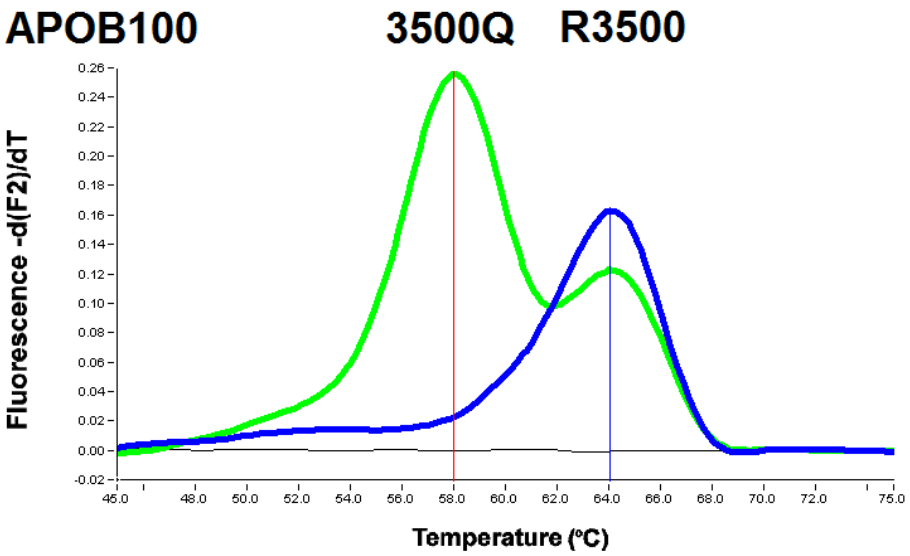

Figure 1 : Melting curve analysis of amino acid 3500 R and Q genotypes of APOB100.

BLUE : Homozygote for the RR allele (**wild type** Control contained in the ToolSet, Control APOB-3500).
GREEN : R / Q Heterozygote.
Conditions : LC Program 3.5 with Color compensation and Digital Filter enabled,
Calculation Method : Polynomial, Degrees to Average : 9.
Red Cursor : T_m = 58.1 ^{0}C, Blue Cursor : T_m = 64.1 ^{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 APOB100-3500 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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