ApoE ToolSet™ for LightCycler™
(ApoE E2/E3/E4, codons 112 Cys/Arg & 158 Cys/Arg)

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

Order#: ApoE - 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
			ApoE - 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 ApoE E2/E4 HET DNA	Dissolved: 20 µL
3, Blue cap	**Solvent**	- to dissolve OligoTool / Control	1000 µL of Solvent
4, Yellow Cap	**DMSO** **Caution Irritant !** **May be absorbed through skin !**	- PCR additive for GC rich regions	100 µL of DMSO Note : Fluid above 18 °C Solid below 18 °C

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 multiplex genotyping of the codon 112 Cys / Arg and codon 158 Cys / Arg polymorphisms in the Apolipoprotein E (ApoE) gene, encoding the **E2/E3/E4 isoforms of Apolipoprotein E**, by LightCycler PCR with Melting Curve Analysis. Primers and fluorescent probes have been optimized for specific amplification of a 462 bp segment containing the relevant site and for optimal genotype discrimination.

Control material E2 / E4 Heterozygous Control DNA, lyophilized
(= Cys112Arg / Cys158Arg compound heterozygote).

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 **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 ApoE -16, dissolved	2.8
Solvent ApoE -16	8.0
$MgCl_2$ 25 mM	1.2 (final 2.5 mM)
DMSO (yellow cap)	2.0
Master Hybridization Probes 10x	2.0
Total Reaction Mix	16.0
+ Your DNA or Control ApoE E2/E4 HET –16	4.0
Grand Total	20.0

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 ApoE E2/E4 HET - 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 **ApoE** ToolSet™ for LightCycler™ allows detection of the **codon 112 Cys/Arg and codon 158 Cys/Arg variants** in the ApoE gene, encoding the **E2/E3/E4 isoforms of Apolipoprotein E**.

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)	60
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	**60**		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**58**	72
Incubation time (s)	**5**	**10**	**15**
Temperature Transition Rate (°/s)	20	20	**3**
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	**30**	0
Temperature Transition Rate (°/s)	20	20	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
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 with automatic gain control.
For readout use F2 and F3 **with color compensation (obligatory !)**.

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 codon 112 and 158 polymorphisms in the ApoE gene. Figure 1 shows a typical result obtained with the ApoE ToolSet™ for LightCycler™ :

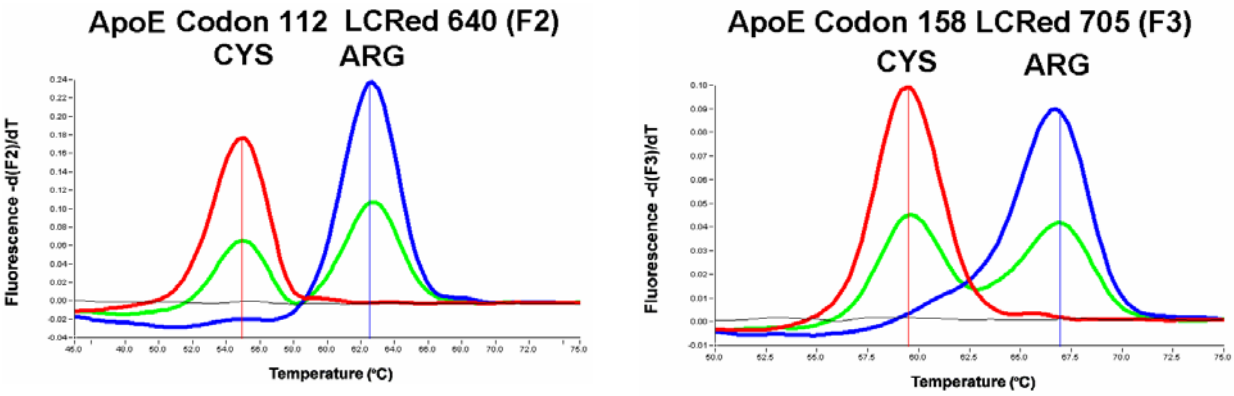

Figure 1 : Melting curve analysis of genotypes of codons 112 and 158 in the ApoE gene.

BLUE : Homozygotes **Arg112Arg** (left) and **Arg158Arg** (right)
GREEN : Compound Heterozygote **Cys112Arg / Cys158Arg** = ApoE E2 / E4 HET Control contained in the ToolSet.
RED : Homozygotes **Cys112Cys** (left) and **Cys158Cys** (right)
BLACK : No DNA Control.

Conditions : LC program version 3.5, Color Compensation and Digital Filter enabled, Degrees to average : 8, Calculation Method : Polynomial

For conversion of codon 112 / 158 melting curves into ApoE genotypes, use the following Table :

Table 1 : Conversion of Codon 112 / Codon 158 melting curve results into ApoE genotype
YES : peak observed at indicated Tm *no* **: No peak observed at indicated Tm**

	Codon 112 (LCRed 640, F2)		**Codon 158** (LCRed 705, F3)		
Genotype	**Tm 54 - 56 C**	**Tm 62 - 64 C**	**Tm 59 - 61 C**	**Tm 66 – 68 C**	**Genotype**
E2/E2 (Cys/Cys)	**YES**	*no*	**YES**	*no*	**E2/E2 (Cys/Cys)**
E3/E3 (Cys/Arg)	**YES**	*no*	*no*	**YES**	**E3/E3 (Cys/Arg)**
E4/E4 (Arg/Arg)	*no*	**YES**	*no*	**YES**	**E4/E4 (Arg/Arg)**
E2/E3	**YES**	*no*	**YES**	**YES**	**E2/E3**
E2/E4	**YES**	**YES**	**YES**	**YES**	**E2/E4**
E3/E4	**YES**	**YES**	*no*	**YES**	**E3/E4**

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 ApoE 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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