ratiogen

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For *IN VITRO* USE ONLY. Version 1, February 2025

α1 Antitrypsin PI-S ToolSet™ for LightCycler™
Application on LightCycler 480

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: α1AT PI-S - 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 Fast Start DNA Master HybProbes, 10 x conc. (Roche Cat.No.: 03003248001) and LightCycler® 480 Multiwell Plates 96, white (Roche Cat.No.: 04729692001)

1. ToolSet contents

Vial	Label	Content	Quantity
			α1AT PI-S - 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 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® Fast Start DNA Master HybProbes, 10 x conc.Cat.No.: 03003248001, including 25mM $MgCl_2$;
LightCycler® 480 instrument, LightCycler® 480 Multiwell Plates 96 white, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description

The ToolSet is specifically adapted for genotyping the PI-S variant of α1 Antitrypsin (Glu264Val, A9628T, Gene-Bank Accession No. K02212) 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 ?
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 α1AT PI-S dissolved	2.8
Solvent α1AT PI-S	9.6
$MgCl_2$ 25 mM	1.6 (final 3mM)
FastStart DNA Master HybProbe, 10x	2
Total Reaction Mix	16
+ Your DNA or Control α1AT PI-S	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 heterozygous Control α1AT PI-S 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 α1AT PI-S ToolSet™ for LightCycler™ allows the detection of the PI-S variant of α1 Antitrypsin (Glu 264Val) which is associated with low plasma levels of α1 Antitrypsin.

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

Note : This ToolSet employs the same LightCycler Time-Temperature protocol (next page) as the α1AT PI-**Z** ToolSet and can be used in the same run.

4. LightCycler 480 Settings and Experimental Protocol

For use with LC 480 Program Version 1.5 series.
Detection : Dynamic Red 640 (498-640 nm)

Fast Start Enzyme Activation and DNA Denaturation

Cycle Program Data	**Value**
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature (°C)	**95**
Incubation time (s) **Hold**	**600**
Temperature Transition Rate (°C/s) **Ramp Rate**	**4.4**
Acquisitions (per °C)	---
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

Amplification

Cycle Program Data	**Value**		
Cycles	**35**		
Analysis Mode	Quantification		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	**95**	**53**	**72**
Incubation time (s) **Hold**	**5**	**10**	**15**
Temperature Transition Rate (°/s) **Ramp Rate**	**4.4**	**2.2**	**4.4**
Acquisitions (per °C)	---	---	---
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) **Hold**	**60**	**60**	---
Temperature Transition Rate (°/s) **Ramp Rate**	**4.4**	**2**	**0.14**
Acquisitions (per °C)	---	---	**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) **Hold**	**30**
Temperature Transition Rate (°/s) **Ramp Rate**	**1.5**
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

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 α1AT PI-S ToolSet™ for LightCycler™ :

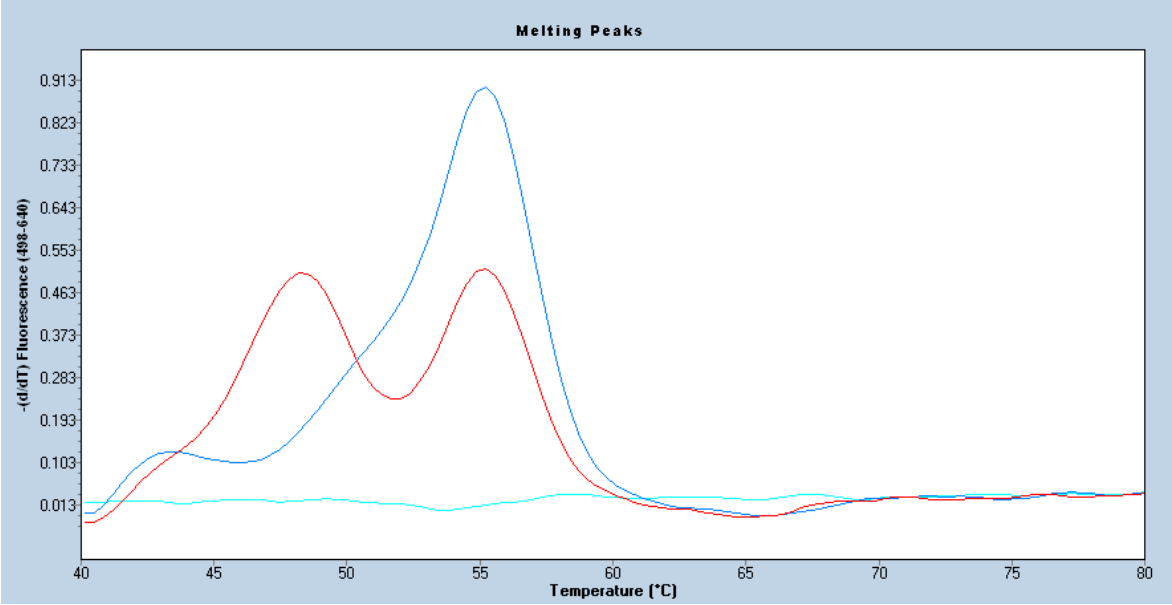

Figure 1 : Melting curve analysis of amino acid 264 PI-M and PI-S genotypes of α1AT.

BLUE : Homozygote **MM wild type** ($T_{m(}$ (**M**)= 55,4 °C).
RED : Heterozygote **MS mutation** Control contained in the kit (T_m (**S**) = 48,3 °C).
Light Blue : No DNA Control.

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Conditions : LC 480 program version 1.5

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 α1AT PI-S 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

The purchase of this product does not convey any right for its use in clinical diagnostics.
No license to use the PCR process is conveyed expressively or by implication by purchase of this product.
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