ratiogen

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For *IN VITRO* USE ONLY. Version 2, March 2025

ALDOB N334K ToolSet™ for LightCycler™
(Aldolase B, Hereditary Fructose Intolerance)

Application on LightCycler 480

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: ALDOB 334 - 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
			ALDOB 334 - 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® 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 This ToolSet is specifically designed for genotyping the N334K polymorphism (C3925G at nt level) in the Aldolase B (ALDOB) gene by LightCycler PCR with Melting Curve Analysis. Primer pair and fluorescent detection and anchor probes have been optimized for specific amplification of a 119 bp segment containing the potentially mutated site 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 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 ALDOB 334 -16, dissolved	2.8
Solvent ALDOB 334 -16	8.8
$MgCl_2$ 25 mM	**2.4 (final 4 mM)**
Fast Start DNA Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control ALDOB 334 –16	4
Grand Total	20

Use Fast Start DNA Master Hybridization Probes 10x and $MgCl_2$ 25 mM from Roche LightCycler Fast Start DNA Master Hybridization Probes, 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 ALDOB NN334 wild type 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 **ALDOB N334K** ToolSet™ for LightCycler™ allows detection of the **C→G** mutation at position 3925 in the ALDOB gene resulting in the **Asn 334 Lys** exchange in the Aldolase B protein causing a **loss of enzymatic activity**. **The homozygous 334 Lys mutant of Aldolase B is a frequent cause of Hereditary Fructose Intolerance**.

Note : In parts of the scientific literature this mutation is also designated as N335K due to different amino acid numbering.

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

Note : This ToolSet uses the same Time-Temperature Protocol as the ALDOB A149P and ALDOB A174D ToolSets and can therefore 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) with Colour Compensation

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**	**55**	**72**
Incubation time (s) **Hold**	**5**	**10**	**10**
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**	**85**
Incubation time (s) **Hold**	**30**	**60**	---
Temperature Transition Rate (°/s) **Ramp Rate**	**4.4**	**1.5**	**0.29**
Acquisitions (per °C)	---	---	**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) **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 possible genotypes at the **N334K** mutation site in the **ALDOB** gene. Figure 1 shows a typical result obtained with the **ALDOB N334K** ToolSet™ for LightCycler™ :

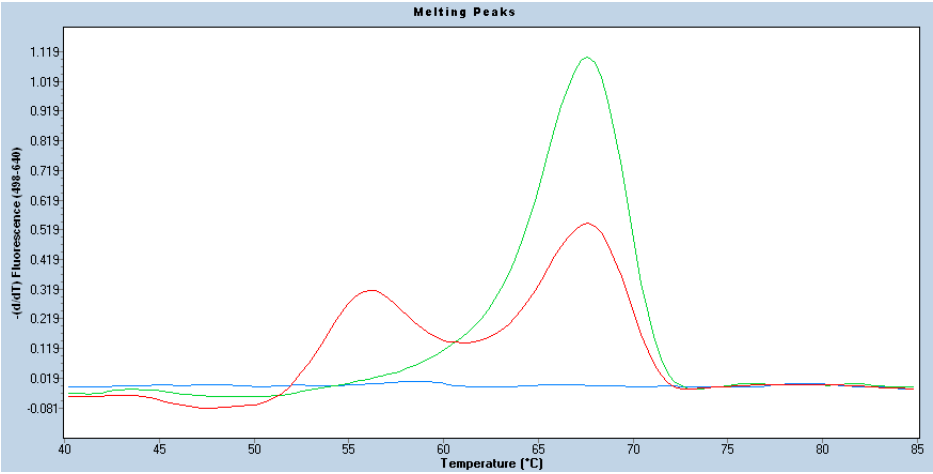

Figure 1 : Melting curve analysis of genotypes at AA position 334 of the human ALDOB gene

GREEN : **Homozygote NN334** (T_m (**NN**) = 67.2 °C, wild type Control DNA contained in the ToolSet)
RED : **Heterozygote N334K** (T_m (**N**) = 67.2 °C, T_m (**K**) = 56.9 °C)
BLUE : No DNA Control

Conditions : LC 480 program version 1.5, with Colour compensation,

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 ALDOB N334K 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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No license to use the PCR process is conveyed expressively or by implication by purchase of this product.
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How to contact ratiogen

E-mail info@ratiogen.com

Internet http://www.ratiogen.com