Protocol uses Fast Start DNA Master SYBR Green !

ratiogen

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HLA DQA*05 ToolSet™ for LightCycler™
(HLA DQ2, PCR, Celiac Disease, Zöliakie)

Lyophilized ToolSet for PCR using the LightCycler™ Instrument.

Order#: HLA DQA*05 - 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 SYBR Green, 10 x conc. (Roche Cat.No.: 03003230001)

1. ToolSet contents

Vial	Label	Content	Quantity
			HLA DQA*05
1, Red cap	**OligoTool**	- lyophilized oligos for PCR - contains a primer set for HLA DQA*05 and a primer set for α1-AT Pi-Z as amplification control	For 16 tests Dissolved: 50 µL
2 A–B , Green caps	**2 Controls**	- **A** : lyophilized HLA DQA*05 **positive** DNA - **B** : lyophilized HLA DQA*05 **negative** 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 SYBR Green, 10 x conc.Cat.No.: 03003230001, 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 detecting presence of the HLA DQA*05 allele of the human HLA DQ locus by LightCycler PCR with Melting Curve Analysis. Co-amplification of a sequence of the human α1-AT gene serves as internal positive control. The two primer pairs have been optimized for specific amplification of HLA DQA*05 (positive if present, yielding a 205 bp segment) and α1-AT (a 136 bp segment in absence of HLA DQA*05, but suppressed in presence of HLA DQA*05).

Control material - **A** : HLA DQA*05 **positive** DNA, - **B** : HLA DQA*05 **negative** DNA, both 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.

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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 extra primers, they are already contained in the OligoTool.
You don't have to add probes, this setup does not require probes.

Reaction Mix Preparation

For 1 (One) reaction, prepare the Reaction Mix as shown in the following table :

Reagent	µL
OligoTool HLA DQA*05, dissolved	2.8
Solvent HLA DQA*05	9.6
$MgCl_2$ 25 mM	1.6 (final 3 mM)
Fast Start DNA Master SYBR Green 10x	2
Total Reaction Mix	16
+ Your DNA or Controls HLA DQA*05	4
Grand Total	20

Use Fast Start DNA Master SYBR Green 10x and $MgCl_2$ 25 mM from Roche LightCycler DNA Master SYBR Green, 10 x conc.
(Roche Cat.No.: 03003230001, including 25mM $MgCl_2$).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control

Always run positive controls with the samples. Use the dissolved Controls **A** : **HLA DQA*05 positive DNA** and **B** : **HLA DQA*05 negative DNA** Both contained in the ToolSet (Green Caps).

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 **HLA DQA*05** ToolSet™ for LightCycler™ allows the detection of the **DQA*05 allele** of the HLA-DQ locus associated with Coeliac Disease (CD) : 90 % of CD patients express the DQA*05 – DQB*02 heterodimer (serologically HLA DQ2). The remaining 10 % carry the DQA*03 – DQB*03:02 heterodimer (serologically HLA DQ8).
The above alleles are relatively frequent in the general population - e.g. DQB*02 : ~ 30 %- which is far greater than the 1 % worldwide prevalence of CD. Thus the positive predictive value of these alleles for the development of CD is low pointing to the contribution of other genes and environmental factors.
However, absence of the DQA*05 allele together with absence of the DQB*02 and DQB*03:02 alleles has a near perfect negative predictive value for Coeliac Disease.
(Wolters VM et al. 2008, PubMedID : 18184122 , Husby S et al. 2012, PubMed ID 22197856)

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

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4. LightCycler Settings and Experimental Protocol

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)	**600**
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	**45**		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**70**	**72**
Incubation time (s)	**2**	**5**	**10**
Temperature Transition Rate (°/s)	20	**20**	**20**
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	None	**Single**

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	**70**	**99**
Incubation time (s)	30	30	0
Temperature Transition Rate (°/s)	20	20	**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
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.

For readout use channel **F1** (Fluorescein) resp. **SybrGreen I / HRM 465 – 510 nm**

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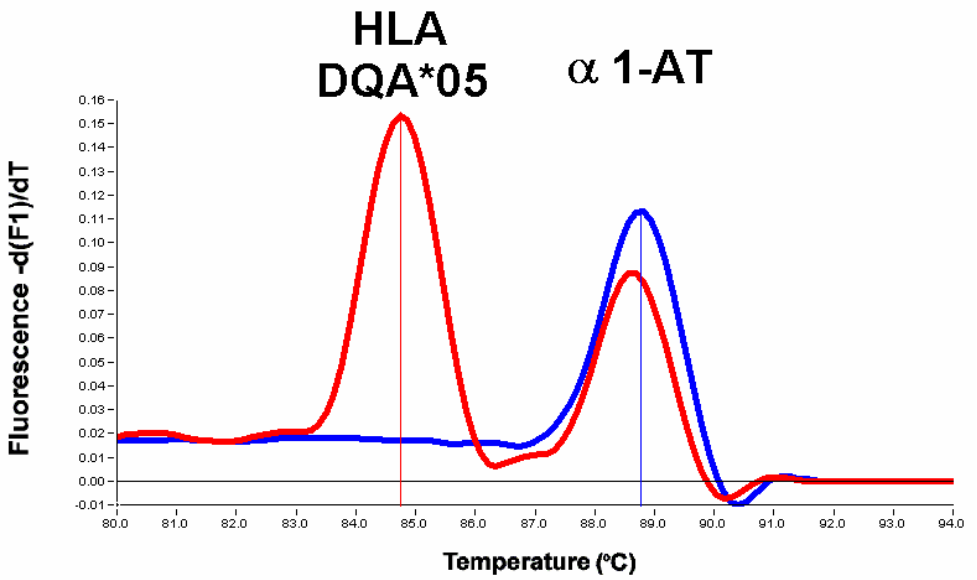
5. Typical results

Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples. The melting peaks allow discrimination between presence or absence of the HLA DQA*05 allele with the Factor II amplicon peak serving as positive control. The Figure shows a typical result obtained with the HLA DQA*05 ToolSet™ for LightCycler™ :



Melting curve analysis of HLA DQA*05 and α1-AT amplicons.

BLUE : **HLA DQA*05 negative** Control with α1-AT amplicon only
RED : **HLA DQA*05 positive** Control with DQA*05 and α1-AT amplicons
BLACK : No DNA Control

Blue Cursor (α1-AT): T_m = 88.7 °C **, Red Cursor** (HLA DQA*05): T_m = 84.7 °C.

Note : **In presence of HLA DQA*05, amplification of α1-AT is variably suppressed.**

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation, Digital Filter enabled, Calculation Method : Polynomial, Degrees to average : 4.

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 HLA DQA*05 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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