

Filaggrin R501X ToolSet™ for LightCycler™ (Ichthyosis, Atopy, Allergy, Asthma, Eczema, FLG, PCR)

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

Order#: FLG 501 - 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
			FLG 501 - 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-DNA Master Hybridization Probes, 10 x conc.Cat.No.: 2 015 102, including 25mM MgCl₂; LightCycler
instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description The ToolSet is specifically adapted for genotyping the **R501X variant of Filaggrin** (FLG, Gene-Bank Accession No. NT 004487, Palmer et al 2006, PubMed ID 16550169) by LightCycler PCR with Melting Curve Analysis. Fluorescent detection and anchor probes and the primer pair have been optimized for specific amplification 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 ? 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 FLG 501, dissolved	2.8
Solvent FLG 501	9.6
MgCl ₂ 25 mM	1.6 (final 3 mM)
FastStart DNA Master HybProbe, 10x	2
Total Reaction Mix	16
+ Your DNA or Control FLG 501	4
Grand Total	20

Use FastStart DNA Master HybProbe 10x and MgCl₂ 25 mM from Roche LightCycler FastStart DNA Master HybProbe, 10 x conc. (Roche Cat.No.: 03003248001, including 25mM MgCl₂).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control Always run a positive control with the samples.
Use the dissolved heterozygous Control FLG R501X 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 **FLG R501X** ToolSet™ for LightCycler™ allows the detection of the **R501X mutation of the Filaggrin gene** which causes a premature stop and is associated with Ichthyosis, Atopy, Allergy, Asthma and Eczema.

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)	900
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	60		
Analysis Mode	None		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	60	72
Incubation time (s)	10	5	8
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	0.1
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)	20.0
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 FLG R501X ToolSet™ for LightCycler™ :

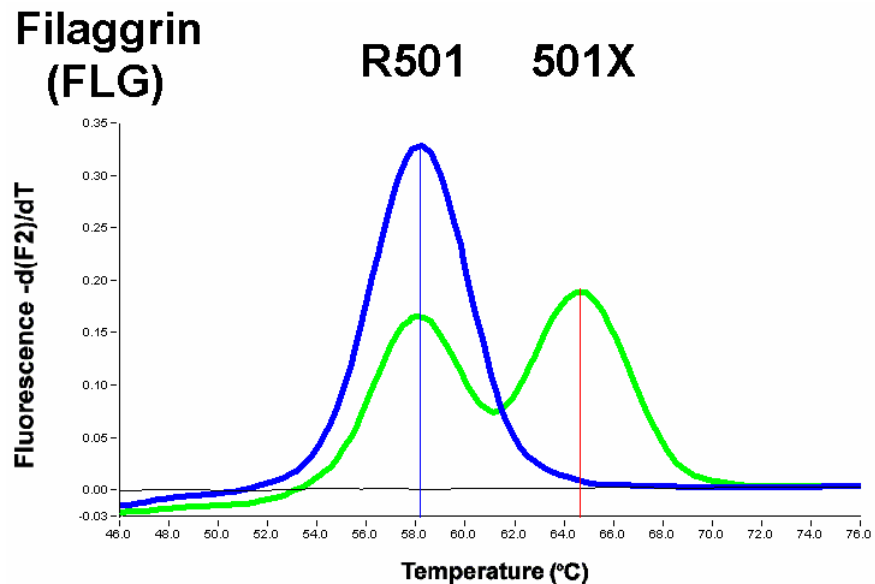


Figure 1 : Melting curve analysis of WT and R501X genotypes of the FLG gene.

BLUE : Homozygote wild type, RR 501.

GREEN : Heterozygote R501X Control contained in the ToolSet, Control FLG R501X HET.

BLACK : No DNA Control

Conditions : LC Program 3.5 without Color compensation but Digital Filter enabled,

Calculation Method : Polynomial, Degrees to Average : 10.

Red Cursor : $T_m = 64.6$ °C, Blue Cursor : $T_m = 58.2$ °C

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 +/- 2.0 °C. The FLG R501X 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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How to contact ratiogen

E-mail info@ratiogen.com

Internet <http://www.ratiogen.com>