

FcγRIIIA V158F ToolSet™ for LightCycler™ (at nucleotide level : G559T)

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

Order#: FcgRIIIa 158 - 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 |
|---------------------|------------------|---|--------------------------------------|
| | | | FcgRIIIa 158 - 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 VF 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 SYBR Green, 10 x conc., Cat.No.: 03003230001, including 25mM MgCl₂; LightCycler instrument & capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description This ToolSet is specifically designed for genotyping the V158F polymorphism in the FcγRIIIa gene by multiplex allele-specific LightCycler PCR with Melting Curve Analysis. Primers are optimized for the specific amplification of ≈ 70 bp (V allele) and ≈ 120 bp segments (F allele) allowing optimal genotype discrimination.

Control material Heterozygous V158F 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. 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 FcγRIIIa 158-16, dissolved | 2.8 |
| Solvent FcγRIIIa 158 -16 | 11.4 |
| MgCl ₂ 25 mM | 0.8 (final 2 mM) |
| Fast Start DNA Master SYBR Green 10x | 2 |
| Total Reaction Mix | 17 |
| + Your DNA or Control FcγRIIIa V158F -16 | 3 |
| Grand Total | 20 |

Use Fast Start DNA Master SYBR Green 10x and MgCl₂ 25 mM from Roche LightCycler DNA Master SYBR Green, 10 x conc. (Roche Cat.No.: 03003230001, including 25mM MgCl₂).
 For multiple reactions, multiply the indicated volumes appropriately.

Positive Control Always run a positive control with the samples. Use the dissolved FcγRIIIa V158F heterozygous 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 **FcγRIIIa V158F** ToolSet™ for LightCycler™ allows detection of the **G→T** single nucleotide polymorphism at position 559 in the FcγRIIIa gene resulting in the **Val158Phe (V158F)** exchange in the FcγRIIIa protein. The V/V genotype results in a more efficient binding of IgG1 or IgG3 to NK cells and in an increased NK cell activation by aggregated IgG as compared to the F/F genotype.
The FcγRIIIa V158F polymorphism has shown associations to **immune-related disorders** (e.g. lupus erythematoses, rheumatoid arthritis, Wegener's granulomatosis, adult periodontitis, HIV-related Kaposi's sarcoma) and may influence the response to therapeutically used antibodies.

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

4. LightCycler Settings and Experimental Protocol

Denaturation and FastStart Enzyme Activation

| 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 | 43 | | |
| Analysis Mode | None | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 3 |
| Target Temperature (°C) | 95 | 56 | 72 |
| Incubation time (s) | 10 | 10 | 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 | 60 | 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 and automatic gain control.
For readout use channel F1(Fluorescein).

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 **V158F** polymorphism in the **FcγRIIIa** gene. Figure 1 shows a typical result obtained with the **FcγRIIIa V158F ToolSet™** for LightCycler™ :

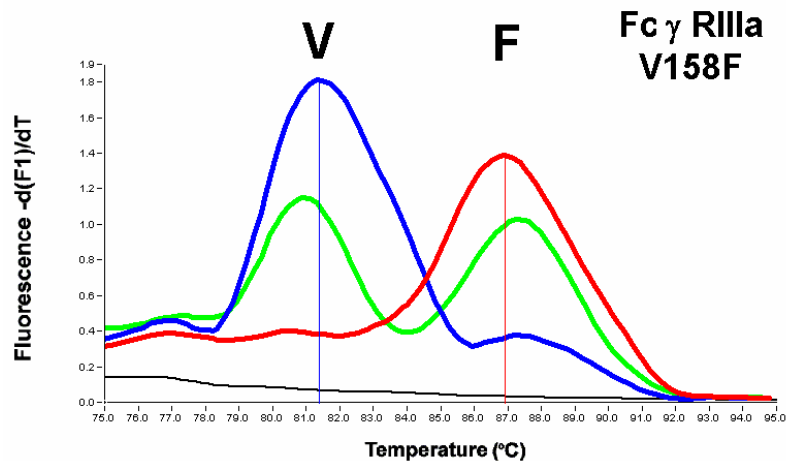


Figure 1 : Melting curve analysis of possible genotypes at position 158 of the human FcγRIIIa gene

BLUE : Homozygote **VV 158** DNA ("Val Val" genotype)
GREEN : Heterozygote **V 158 F** Control DNA contained in the ToolSet ("Val Phe" genotype),
RED : Homozygote **158 FF** DNA ("Phe Phe" genotype)
BLACK : No DNA Control.

Blue Cursor : $T_m = 81.4\text{ }^{\circ}\text{C}$; **Red Cursor :** $T_m = 86.9\text{ }^{\circ}\text{C}$

Note : Heterozygotes show slightly larger T_m difference between peaks than Homozygotes.

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation, Digital Filter enabled, Degrees to average : 8.0. Calculation Method : Polynomial.

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 FcγRIIIa V158F 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>