

LightMix[®] Kit *IL6 G-174C* Cat.-No. 40-0309-16

Kit with reagents for the detection of the *human IL6 G-174C* DNA polymorphism using the LightCycler[®] 1.x / 2.0 Instruments.

Lyophilized mix of primers and probes (6 tubes with 16 rxns each) for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

Additional reagents required

Roche Diagnostics:

LightCycler[®] FastStart DNA Master HybProbe

Cat.-No. 03 003 248 001

High Pure PCR Template Preparation Kit

Cat.-No. 11 796 828 001

1. Introduction

IL6 (Interleukin 6, OMIM*147620) is a highly controlled cytokine and is one of the most important regulators of the inflammatory process. IL6 stimulates the immune response and is a key factor in the formation of acquired immunity¹.

The *IL6 G-174C* polymorphism, where in general the -174C variant is associated with lower IL6 secretion², is involved in the development and the course of several diseases, e.g. Kaposi sarcoma³ (statistically overrepresented: -174G), insulin-dependent diabetes mellitus⁴ (risk variant: -174C), Crohn disease⁵ (growth retarding: -174G).

The LightMix[®] Kit *IL6 G-174C* provides a fast, easy and accurate system to identify the genotype of this target in a nucleic acid extract.

This LightMix[®] Kit is tested with the Roche Diagnostics 'LightCycler[®] FastStart DNA Master HybProbe' in the LightCycler[®] 1.x / 2.0 Instruments.

¹Directing Transition from Innate to Acquired Immunity: Defining a Role for IL-6. Jones SA. *J Immunol* **175**:3463-8 (2005).

²The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. Fishman D et al. *J Clin Invest* **102**:1369-76 (1998).

³An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Foster CB et al. *Blood* **96**:2562-7 (2000).

⁴Association of a functional 17-beta-estradiol sensitive IL6-174G/C promoter polymorphism with early-onset type 1 diabetes in females. Kristiansen OP et al. *Hum Molec Genet* **12**:1101-10 (2003).

⁵Intestinal inflammation-induced growth retardation acts through IL-6 in rats and depends on the -174 IL-6 G/C polymorphism in children. Sawczenko A et al. *Proc Nat Acad Sci* **102**:13260-65 (2005).

2. Description

A 175 bp fragment of the human *IL6* gene spanning the promoter *IL6 G-174C* region is amplified with specific primers. The resulting PCR fragments are analyzed with hybridization probes labeled with LightCycler[®] Red 640. The genotype is identified by running a melting curve with specific melting points (T_m). The wildtype allele *IL6 G-174* exhibits a T_m of 64.0°C in channel 640. The allele variant *IL6 -174C* exhibits a T_m of 57.0°C in channel 640.

For use in LightCycler[®] 1.x Instruments use channel F2 instead of channel 640 for detection.

3. Set contents

- 6 Vials with red caps containing premixed lyophilized primers and probes for 16 PCR reactions each of *IL6 G-174C*

4. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:		Denaturation	Cycling			Melting			Cooling
Settings LC 1.x/2.0	Parameter								
	Analysis Mode	None	Quantification mode			Melting Curves mode			None
	Cycles	1	45			1			1
	Target [°C]	95	95	60	72	95	40	85	40
	Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
	Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
	Acquisition Mode	None	None	Single	None	None	None	Continuous	None

5. Data analysis

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

View *IL6 G-174C* data in channel 640 "Tm Calling" Analysis mode (LightCycler® 2.0 Instrument) or Melting Curves mode (LightCycler® 1.x Instrument). The negative control (NTC) must show no signal.

6. Product characteristics

PCR results are obtained within 1 hour.

Sensitivity

These reagents detect 1 ng of human genomic DNA.

Measuring range

The measuring range of the assay is 1 ng to 100 ng of human genomic DNA.

Storage and Stability

- Lyophilized reagents are stable for at least 3 months after shipment if stored protected from light at room temperature (18-25°C).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days if stored protected from light and refrigerated (4°C).

7. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 Instruments. Start programming before preparing the solutions. See the LightCycler® Instrument operator's manual for details.

Sample material: Use aqueous nucleic acid preparations (e.g. Roche Diagnostics 'High Pure PCR Template Preparation Kit').

Negative control: Always run at least one negative control - replace the template DNA with water.

7.1. Preparation of parameter-specific reagents (16 reactions):

One reagent vial with a **red** cap contains all primers and probes to run 16 LightCycler® reactions for *IL6 G-174C*.

Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► Use 4 µl **reagent** for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

7.2. Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart Master	
Single reaction	Component
7.4 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
1.6 µl	Mg ²⁺ solution 25 mM (blue cap, provided with the Roche FastStart kit)
4.0 µl	reagent mix (parameter specific reagents containing primers and probes, see 7.1.)
2.0 µl	Roche Master (red cap, for preparation see Roche manual)

15.0 µl

Volume of reaction mix

Mix gently, spin down and transfer 15 µl each of the reaction mix to a LightCycler® capillary.

Add 5 µl of sample to each capillary for a final reaction volume of **20 µl**.

Start run.

8. Sample data - typical results

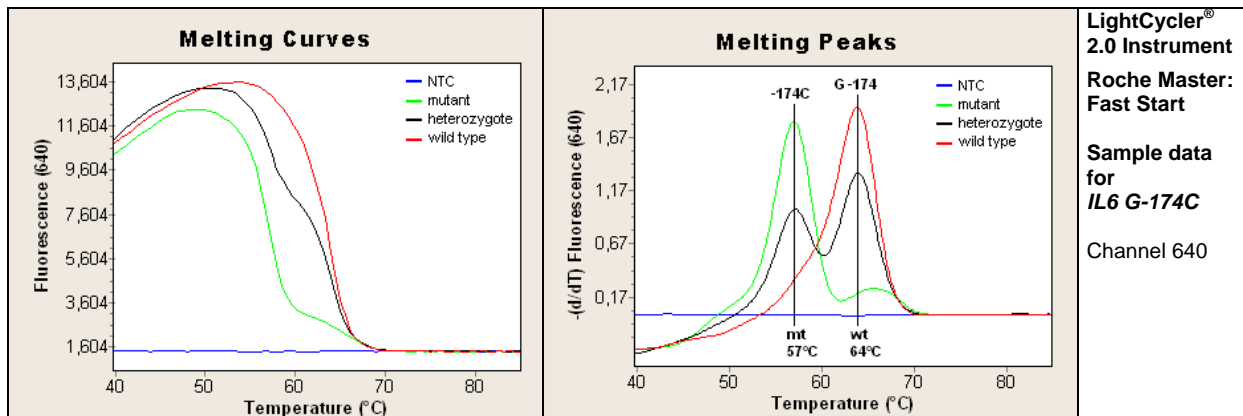


Fig.1. Sample data for the IL6 G-174C detection system.

Data from LightCycler® 2.0 Instrument. Left panel channel 640 melting curves for IL6 G-174C Right panel channel 640 melting peaks for IL6 G-174C . Wildtype (wt) corresponds with IL6 -174G/G, heterozygote corresponds with IL6 -174G/C and mutant (mt) corresponds with IL6 -174C/C.

9. Interpretation of data

Genotype:	mutant homozygote IL6 -174C/C	heterozygote IL6 -174G/C	wild type homozygote IL6 -174G/G
Number of melting peaks (color)	1 (green)	2 (black)	1 (red)
Melting temperature of peaks	57.0°C	57.0°C and 64.0 °C	64.0°C
Temperature difference between peaks	---	7.0°C	---

Tab. 3. Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)

Notes: The values of the respective melting temperatures (T_m) may vary $\pm 2.5^\circ\text{C}$ between different experiments. The ΔT between the melting peaks for heterozygote genotypes may vary $\pm 1.5^\circ\text{C}$. Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact service@tib-molbiol.de).

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