

8. Sample Data - Typical Results

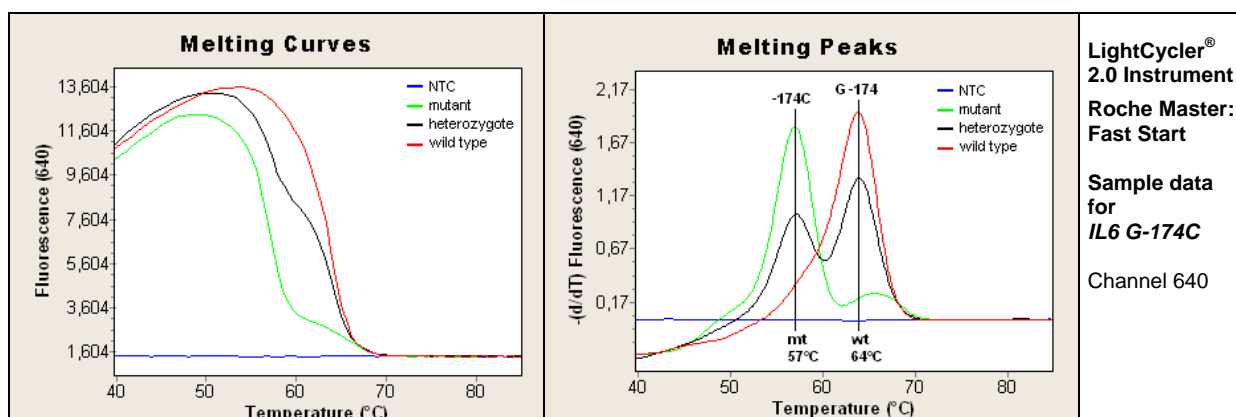


Fig.1. LightCycler® 2.0 Instrument sample data for the *IL6 G-174C* detection system.

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 <i>IL6 -174C/C</i>	heterozygote <i>IL6 -174G/C</i>	wild type homozygote
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	---

Table 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).

10. Material Safety Data

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008 (1999)] and the European Union Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

11. Version History

Notes in red mark events require to change procedures

V120321 Previous version
V130913 Editorial changes

Roche SAP order n° 05945585001

Notice to Purchaser

The purchase price of this product includes limited, nontransferable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. The purchase of this product does not convey any right for its use in clinical diagnostic applications.

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany. LightCycler® hybridization probes produced under license from Roche Diagnostics GmbH.



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

Kit with reagents for detection of the *human IL6 G-174C* DNA polymorphism using Roche Diagnostics 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
LightCycler [®] Capillaries (20 µl) (LightCycler [®] 1.x / 2.0 Instruments)	Cat.-No. 04 929 292 001

1. Introduction

Interleukin-6, (IL6, 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.

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

² The 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).

³ An 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).

⁴ Association 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).

⁵ Intestinal 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 with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler[®] 1.x Instruments to software version 4.1.

3. Set Contents

- 6 Vials with **red** cap containing lyophilized primers and probes for 16 PCR reactions *IL6 G-174C*
- 1 Vial with **colorless** cap containing control DNA (IL6 wt G-174)
- 1 Vial with **colorless** cap containing control DNA (IL6 mt -174C)
- 1 Vial with **colorless** cap containing control DNA (IL6 het)

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

Table 1

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 6 months after shipment when stored protected from light at room temperature (18-25°C). See expiry date on the product label.
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days when 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 Instrument operator's manual for details.

Sample material: Use aqueous nucleic acid preparations (e.g. 'High Pure PCR Template Prep. 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 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 control DNA

Add 80 µl PCR-grade water to each vial (1.6×10^6 target molecules). Mix the target DNA by pipetting the solution up and down 10 times.

Note: Control DNA can be dissolved up to 160 µl to achieve 32 control reactions.

► Use 5 µl control DNA for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C, for long term storage freeze at -20°C, avoid repeated freezing thawing cycles.
Please note that opening of these vials may cause contaminations of the work-space (aerosol).

7.3. 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

Table 2

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.