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 immunity[1].
The IL6 G-174C polymorphism, where in general the -174C variant is associated with lower IL6 secretion[2], is involved in the development and the course of several diseases, e.g. Kaposi sarcoma[3] (statistically overrepresented: -174G), insulin-dependent diabetes mellitus[4] (risk variant: -174C), Crohn disease[5] (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 (Tm). The wildtype allele *IL6 G-174* exhibits a Tm of 64.0°C in channel 640. The allele variant *IL6 -174C* exhibits a Tm 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
	Parameter								
	Analysis Mode	None	Quantification mode			Melting Curves mode			None
	Cycles	1	45			1			1
Settings	Target [℃]	95	95	60	72	95	40	85	40
LC 1.x/2.0	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 [℃/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℃).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days if stored protected from light and refrigerated (4℃).

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

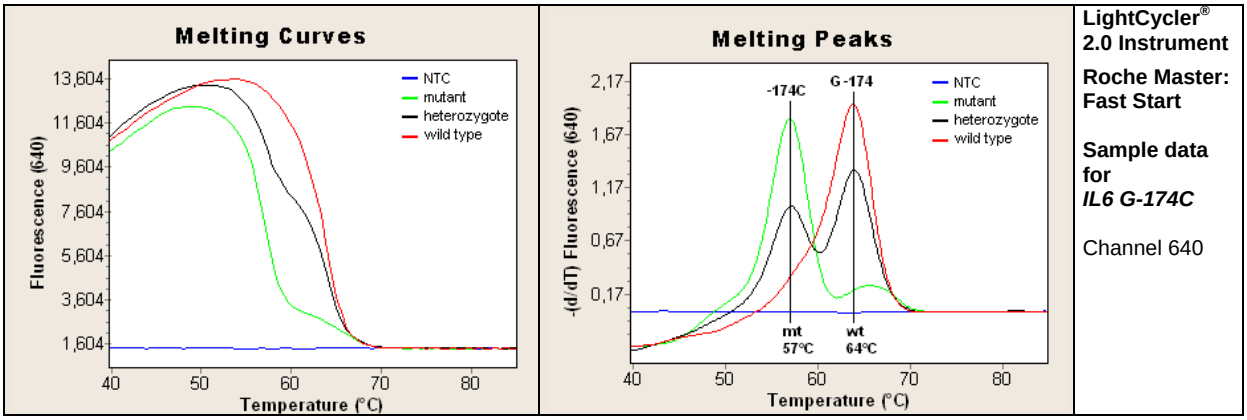

Fig.1. Sample data for the *IL6 G-174C* detection system.

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℃	57.0℃ and 64.0 ℃	64.0℃
Temperature difference between peaks	---	7.0℃	---

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 ±2.5℃ between different experiments. Th e ΔT between the melting peaks for heterozygote genotypes may vary ±1.5℃. Samples with deviating melting curves sh ould be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact service@tib-molbiol.de).